

Root Development of *Lolium perenne* in Diesel Contaminated Soil

Rotutveckling hos *Lolium perenne* i dieselkontaminerad jord

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Examensarbete (Master Thesis)

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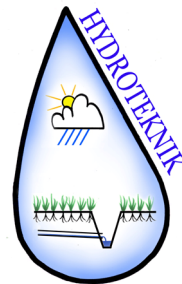
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MSc Thesis

Fulfilled in cooperation between Cranfield University at Silsoe & Swedish University of Agricultural Sciences

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ABSTRACT

When studying phytoremediation, one of the most important aspects is root development, with its associated rhizosphere that improves hydrocarbon degradation. Plant performance of *Lolium Perenne* grown in diesel contaminated soil was studied in a rhizobox experiment where root development could be visually monitored. The effect of layer geometry, irrigation and textural heterogeneity on root development was investigated as well as the relationship between root length and diesel degradation. Adding a thin layer of clean soil on top of a contaminated layer allowed germination but all treatments showed severe stress symptoms, such as reduced shoot and root growth, in the presence of diesel and in response to water stress. The presence of a diesel contaminated layer at five cm depth allowed root development in the uncontaminated surface soil without roots penetrating the contamination. With a thinner layer of clean soil roots did penetrate the contaminated layer but showed reduced root development. Natural attenuation was responsible for most of the degradation with a variation between 78 % and 84 % for all treatments. Root length was positively correlated ($r^2 = 0.52$) to diesel degradation but improved degradation was also dependent on spatial distribution of roots where root presence inside the contaminated layer proved to be important. No diesel movement was found regardless of irrigation technique. When a layer of finer material of varying diesel concentration was imbedded in a coarser sand profile the roots were spreading in this layer regardless of diesel concentration emphasising the importance of optimal moisture and nutrient conditions. The importance of moisture was also shown in the treatments with sub-irrigation where the plants received too little water and died.

REFERAT

En av de viktigaste aspekterna inom fytoremediering är rotutvecklingen med den tillhörande rhizosfären där förhöjd mikrobiologisk aktivitet förbättrar nedbrytningen av kolväten. I denna studie har utvecklingen av *Lolium perenne* som fått växa i dieselkontaminerad jord studerats i ett rhizoboxförsök där rötternas utbredning kunnat observeras visuellt genom en glasskiva. Effekterna av föroreningsgeometri, bevattning och skillnad i textur samt relationen mellan rotlängd och dieselnedbrytning undersöktes. Genom att täcka ett lager med förorenad jord med ett lager ren jord grodde förna i alla försök där dieselkoncentrationen annars skulle ha varit för hög för detta. De uppvisade dock allvarliga stresssymptom så som minskad skott- och rotutveckling i respons till dieselförekomsten och vattenstress jämfört med kontrollerna. Förekomsten av diesel på 5 cm djup tillät rotutveckling i den rena jorden ovanför föroreningen utan att rötterna växte in i det förorenade lagret. Ett tunnare ytlager av ren jord tvingade rötterna att växa in i det underliggande förorenade lagret och växten uppvisade en tydlig negativ påverkan. Bakgrundsnedbrytning stod för den största delen av reduceringen av dieselhalter med en variation på mellan 78 % och 84 % för alla behandlingar. Trots det observerades en positiv, om än svag, korrelation mellan rotutveckling, i form av rotlängd, och nedbrytning av diesel ($r^2 = 0,52$), men förbättrad nedbrytning visade sig inte enbart vara beroende av rotlängd utan även av rumslig utbredning. För att rötterna skulle ha en positiv inverkan på nedbrytningen av kolväten krävdes att dessa hade växt in i det dieselkontaminerade området. Inga förflyttningar av diesel, varken uppåt eller nedåt profilen, kunde påvisas, oberoende av bevattningsteknik. Med ett lager av finare textur av varierande dieselkoncentration inbäddat i en profil med i övrigt grövre material, kunde konstateras att rötterna spreds i lagret med finare material oavsett dieselkoncentration vilket betonar vikten av optimala vatten- och näringsförhållanden. Detta kunde också observeras i behandlingen med bevattning underifrån där rötterna fick för lite vatten och dog.

1. INTRODUCTION

Contaminated land is a growing problem around the world and poses an increasing challenge for remediation. Land is a scarce resource and the environmental impact of contamination stretches over both time and space and has a negative affect on plant communities and wildlife as well as the human population. Soil contaminated with hydrocarbons, especially petroleum products, is a common problem because of the high use and dependence of these products in society. The economic feasibility of a clean up procedure plays a major role in the decision making and there is a multitude of soil remediation techniques for hydrocarbon contaminated sites. Several factors influence the choice of remediation method such as the type of contamination, the size of the area affected, soil conditions, legislation, land use and climate and the cost will vary greatly depending on the technique used (McBride, 1994 and Cunningham et al.,1995).

Commonly hydrocarbon contaminated soils are treated with different types of both in-situ and ex-situ engineering methods. An example of an in-situ remediation technique is soil flushing, used to remediate organic contaminants using different types of flushing agents, such as surfactants, acids or salts. This extracts and moves the contaminant to a zone where it can be removed, usually the ground water (Zhou et al., 2005). Another example of an engineering in-situ remediation technique is the use of a strong oxidising agent such as hydrogen peroxide, which is added to the soil. A rapid chemical decomposition of the organic contaminant takes place. This method is especially useful when the contaminant is very persistent and toxic. Other examples of in-situ remediation methods include soil vapour stripping, air sparging, and thermal desorption (Hamby, 1996).

However, soils are often treated ex-situ by for example different types of soil washing where the soil is processed in a scrubber and the oil is removed from the solid phase to the liquid phase with clean soil as a result (Feng et al., 2001). Other examples of ex-situ techniques are thermal desorption, incineration, air stripping or simply excavation and disposal in landfill (Wood, 1997).

This thesis will focus on the use of plants to remediate soil contaminated with diesel which is a part of the larger concept of phytoremediation. Phytoremediation is defined by Cunningham et al., (1996) as the use of plants to remove, contain or render harmless environmental contaminants. The technique can be an alternative to conventional techniques and is used for in-situ remediation of both soil, sediments, water or even air. Previously, most research on phytoremediation has been concerning heavy metals and hyperaccumulating plants but the interest in phytoremediation of organic contaminants has accelerated in the past decade (Alkorta and Garbisu, 2001). Phytoremediation of hydrocarbons works by stimulating the microbial activity in the soil through the rhizoeffect. Plant roots provide a good environment for microbes which then degrade the hydrocarbon by using it as a food source. An important aspect of phytoremediation is thus to maximise root development (Kaimi et al., 2004). There are many techniques that focus on stimulating the microbial activity in the soil through bioremediation. These can work both individually or in combination with phytoremediation (Bento et al., 2005; Huang et al.; 2005, Sarkar et al., 2005).

Phytoremediation offers a cheap way of decontaminate a hydrocarbon polluted soil. The cost of using phytoremediation can be up to half that of many other techniques and can therefore be used on a large scale where vast areas have been contaminated. The fact that normal

agricultural practices can be used enhances the ease of remediation (Flathman, 1999, Cunningham et al., 1996). Depending on properties of both the contaminant and the soil, the possible mobility of the contamination might be an aspect that needs to be taken into consideration. Plants have a drying effect on the soil by their uptake and transpiration of water which can prevent the contaminant from being washed through the profile and thus from polluting ground water and streams (Hillel, 1982). Because phytoremediation uses green plants as a solar powered pump or catalyst the technique causes minimal disturbance to the environment which makes it more acceptable to the public (Macek et al., 2000).

Despite many advantages of phytoremediation there are limitations as well. The time to remediate a hydrocarbon contaminated site is usually longer when using phytoremediation rather than conventional techniques partly because of the time it takes to establish plants as well as a slower remediation rate. Plant properties can also be a limitation. Firstly, when relying on plants for remediation, the success is greatly dependent on the ability of the plant to grow in the circumstances given, i.e. cope with the toxicity of the contaminant. Secondly, the root length determines the maximum depth possible to remediate. Most plants have the majority of their roots in the upper meter of the soil. This means that if the contamination is any deeper than that, phytoremediation might not be an option, but the mobility of the contaminant also determines the maximum depth of phytoremediation (Cunningham et al., 1996).

Current research is focusing on the remediation potential of plants in terms of total petroleum hydrocarbon (TPH) dissipation and on plant response to TPH toxicity. Germination experiments have investigated the effect hydrocarbons have on seed germination and seedling development since plant establishment is of vital importance in phytoremediation (Adam and Duncan, 1999). Many studies focus on root development in the presence of petroleum products and have established a clear relationship between root growth and TPH dissipation (Hou et al., 2000, Huang et al., 2005). The beneficial effect of the roots is caused by the rhizoeffect, where microorganisms are stimulated by the presence of roots and several studies have aimed at confirming a relationship between microbial activity and the breakdown of hydrocarbons in a contaminated soil (Kaimi et al., 2004; Maila et al., 2004).

Since root development is a crucial factor in phytoremediation, it is important to understand the behaviour of roots in response to the geometry of contamination and soil texture. Finer soil textures have better moisture holding capacity than coarser materials. Heterogeneity in soil texture will therefore in itself have an influence on root behaviour, and the reaction in root growth and distribution when introducing contamination in such a system is important to study. Another aspect of heterogeneity is the spatial distribution of hydrocarbon contamination which is also believed to influence root behaviour. The lack of research in these fields is the cause for this study.

1.1. Aims and Objectives

The aim of this study is to investigate the effect on plant establishment and root development of *Lolium perenne* on a heterogeneously diesel contaminated soil. The study will also explore the relationship between plant performance and root distribution on the breakdown rate of diesel. Similarly to Schwartz et al. (1999) this is done in series of rhizobox treatments where root development and distribution can be visually observed.

The first set of treatments will look at the effect on root growth and behaviour in a soil with the contamination concentrated in a layer with clean soil above and below. This part of the study will aim to answer the following questions:

- Will the roots penetrate the contaminated layer after successful germination?
- Will roots avoid contaminated soil if the layer geometry gives the opportunity?
- Does the thickness of the layer of clean soil above the contamination have an impact on plant performance and root development?

The second set of treatments will study how sub-irrigation influence root development and movement of diesel in the profile compared with irrigation from above.

- How do different irrigation techniques influence plant performance and movement of diesel?

The third set of treatments aims to investigate the effect of textural variation on root behaviour. A layer of finer material of different diesel concentrations is embedded in a sandy profile which gives a change in moisture holding capacity. These treatments are designed to find out the following:

- Will roots seek the moisture and spread in the layer of finer material?
- How does diesel in the layer of finer material affect root distribution?

Finally, the study will examine the relationship between root growth and root distribution on dissipation rate of diesel. The questions the study intend to answer are:

- Can root growth be correlated to the level of diesel degradation?
- How does the spatial distribution of roots affect diesel degradation?

2. BACKGROUND

2.1. Diesel and the environment

2.1.1. Chemistry

Diesel consists of 2 000 – 4 000 individual hydrocarbons with the main structural classes being *n*-alkanes, isoalkanes, cycloalkanes and aromatics (Marchal et al., 2003) and with a range in size from C₉ to C₂₀ (Kroening et al., 2001). The degradability of diesel depends on the molecular structure of its components. Straight chain hydrocarbons (*n*-alkanes) are most readily degradable while cycloalkanes and isoalkanes (branched carbon chains) are typically more recalcitrant (Marchal et al., 2003) and low molecular weight hydrocarbons are more toxic than those with high molecular weight (Chaîneau et al., 1997). The most toxic and persistent components of diesel are poly aromatic hydrocarbons (PAH) (Wang et al., 1990) but their proportion in diesel is generally small, only 3 %. Diesel is hydrophobic and its movement in the soil profile is highly dependent on soil characteristics such as particle size distribution and organic matter content. The hydrophobic characteristics of organic matter bind the diesel and retards movement (Adam et al., 2002). In the process of diesel breakdown, metabolites of different characteristics than the original compound, such as higher toxicity and mobility, can form (Wang et al., 1990).

2.1.2. Toxicity

Diesel is one of the most phytotoxic fuels that are found on contaminated sites. The effects of diesel on plants are many and are dependent on the concentration. Both shoot and root biomass are effected. In an experiment conducted by Hou et al. (2000) the shoot biomass of *Lolium perenne*, grown in soils contaminated with 5 mg/g of diesel, was reduced to more than half compared with an uncontaminated control. The root biomass was not as severely affected but still showed a 20 % reduction.

Establishing a plant cover on diesel contaminated sites can be problematic if the concentration of diesel in the soil is too high to effectively support germination. The levels of diesel that are harmful to plants vary depending on the sensitivity of the plant affected. In a germination experiment conducted by Adam & Duncan (1999) they found that at a diesel concentration of 25 mg/g, the germination rate varied from 10 – 88 % for different grasses while for oil seed rape (*Brassica napus*) the germination rate was 100 %. Even if the plants managed to germinate they still showed serious reductions in shoot and root biomass. The shoot biomass of *B. napus* was only 18 % of that of uncontaminated controls and root biomass 20 %. Other studies has shown that plants (*Populus nigra*, *Salix viminalis*) are serious effected by diesel concentrations of 10 mg/g, with their transpiration inhibited (Trapp et al., 2001). Volatilisation of diesel through the soil does not seem to have any effect on germination rate of *L. perenne* and *Trifolium repens* (Kroening et al., 2001).

Diesel also affects the photosynthesis of the plant by injuring the chlorophyll cells and the photosystems (PS). Even at fairly modest levels of diesel contamination (0.1 - 100 mg/kg) the concentration of chlorophyll decreases log linearly with increasing diesel concentrations.

PS II shows a similar relationship but with an even stronger response. The thylakoid membranes become damaged and PS II is disrupted (Green et al., 1996).

Plants are affected by diesel in secondary ways as well as by the direct effect on photosynthesis. When soils are contaminated with petroleum hydrocarbons, the C:N ratio is increased because of the low amount of nitrogen in the fuel. The microbial biomass in the soil increases as the microbes break down the contamination. When the microorganisms use the carbon as a food source they immobilize soil nitrogen in the process. This will leave less plant available nitrogen and plants will show signs of nutrient deficiency (Adam & Duncan, 2003).

Most of the microbes that break down hydrocarbons in the soil are aerobic and will therefore use the oxygen in the air filled pore spaces as they multiply. This can cause anaerobic conditions which affect the root respiration on plants not adapted to water-logging and the roots suffocate (Cunningham et al., 1996).

2.1.3. Volatile loss

The loss by volatilisation from diesel contaminated soil is generally believed to be very small and is correlated to the amount of organic matter in the soil. Organic matter has a high adsorption capacity to bind especially low molecular weight hydrocarbons which are the fractions easiest volatilised and thus prevents volatilisation compared to soil with low organic matter content (Namkoong et al., 2002). Contrary to this, other research has shown a significant loss of diesel by means of volatilisation (Kroening et al., 2001). A silty loam was contaminated with diesel and the volatile loss was compared with a free surface of diesel. The difference was not significant suggesting that diesel does not bind to particles. The organic content of the soil was 11.2 % which should be high enough to cause some adsorption according to Namkoong et al. (2002). The fraction volatilised first was the low molecular weight which is the fraction easiest adsorbed to organic matter according to Namkoong.

2.1.4. Biodegradability

The amount of diesel that is degraded over time is of course dependent on a lot of environmental factors. In a lysimeter trial with a soil artificially contaminated with 60 mg/g of diesel the concentration had decreased to 10 mg/g after 12 weeks with bioremediation compared with a reduction to 40 mg/g for the control. The bioremediation treatment was aimed at increasing the microbial activity (liming, fertilizing and tilling) (Wang et al., 1990).

2.2. **Diesel and the roots**

2.2.1. Plant uptake

Direct uptake of diesel through plant roots does not seem to be an important remediation pathway for hydrocarbons but uptake and its associated toxicity is dependent on concentration. According to Chaîneau et al. (1997) the analysis of the hydrocarbon content in plant stems and leaves showed no difference between plants grown on fuel oil contaminated

soil and those grown on clean soil. Plants grown on soils with contamination concentrations low enough to allow growth, there is no uptake of hydrocarbons. On heavily contaminated soils however, plants cannot withstand the high concentrations of hydrocarbons which are absorbed and the plants die.

2.2.2. The rhizo-effect

Roots are of vital importance for the remediation of hydrocarbons in a contaminated soil but mainly so because of the associated microorganisms. With increasing soil depth microorganisms, plant roots and soil organic matter tend to decrease. Around the roots is a region which is directly influenced by the roots called the rhizosphere. The rhizosphere contains a great number and diversity of microorganisms carrying out many of the vital functions in the soil such as the cycling of nutrients. The rhizosphere is rich in organic substrates supplied by the roots such as amino acids, sugars, protein and cellulose. The roots release these substrates by leakage, diffusion across membranes or by loss of cells. 2 – 6 % of the carbon from the above ground photosynthesis is lost to the soil (Paul & Clarc, 1996).

In the case of hydrocarbon contamination it is the microorganisms in the soil, and then especially in the rhizosphere, that uses the carbon as a food source and thus breaks down the contaminant. Several studies have shown this relationship to be true. Kaimi et al. (2004) showed a correlation between microbial activity and dissipation rate of TPH as well as between dissipation rate and root growth. In other words, the break down of hydrocarbons is dependent on the activity of microbes in the soil measured as dehydrogenase activity. The presence of plant roots clearly increases the rate of TPH breakdown and this is related to the above ground plant growth. As the plant biomass increases the organic substances produced by the roots also increases which stimulates the microbial population.

There is however microorganisms not only in the rhizosphere but also in the soil matrix. Even during harsh conditions such as draught where the microbial activity is very low, breakdown of TPH still occurs. In a treatment with sterilized soil, which received no irrigation to minimise microbial activity, a 13 % TPH reduction still occurred due to microbial activity (Kaimi et al., 2004).

2.2.3. Root development and dissipation rate

Root development is, as discussed above, important for the breakdown of hydrocarbons because of the microorganisms in the rhizosphere surrounding the roots, but evidence of the beneficial effect of roots does not become clear until the root system has fully developed. This was shown in a pot trial with diesel contaminated soil planted with *L. perenne* conducted by Hou el al. (2000). For the first 45 days there was little difference in TPH reduction between planted and unplanted treatments but when full root establishment had been achieved (102 days), the TPH reduction in pots with grass was 60 % compared with 34 % for unplanted soil.

Similar results were obtained by Kaimi et al. (2004). In a time-course pot experiment the relationship between root growth, microbial activity and dissipation rate for a soil contaminated with diesel was studied. The soil had a diesel content of 18 mg/g and planted

with *L. perenne* and a control without plants was subjected to the same conditions. The result showed that after 91 days TPH levels continued to decrease in planted pots whereas the decrease in unplanted controls levelled off. Up to that point no statistical difference in TPH levels between planted and unplanted pots had been detected. The presence of roots does therefore increase the dissipation of TPH but not until roots have fully developed which in this case was three months.

The size of experimental pot is shown to have a great impact on root growth. With a smaller volume of the experimental pot the root density becomes much larger compared with grass grown in larger pots. These effects are significant in both clean and contaminated soil. The higher root mass density in smaller pots, which in a study by Hou et al. (2001) was twice as high compared with larger pots, causes a relatively higher TPH dissipation. After 84 days there had been a 90 % reduction in the small pots compared with 60 % after 120 days in the larger. This shows how risky it can be to directly implement test results to field scale. An experiment done in small containers would greatly overestimate the effectiveness of the remediation potential of the plant system. (Hou et al., 2001).

3. MATERIALS AND METHODS

3.1. Experimental set up

Two sets of experiments were conducted. The first one consisted of five treatments, replicated three times, all in homogenous soil with a layer of diesel contamination. The treatments were: *Normal* (irrigated from the top), *Sub-irrigation* (irrigated 5 cm below the contaminated layer by four pipes, 4 mm in diameter, drilled through the back), *Discontinuous* (with the contaminated layer shifted down 1.5 cm in the middle with clean soil in between), *Control* (without contamination) and one control with *No Plants* (Figure 3.1). For all but the Discontinuous treatment the layer thicknesses were 1.5 cm layer of diesel contaminated soil covered by 2.5 cm of clean soil.

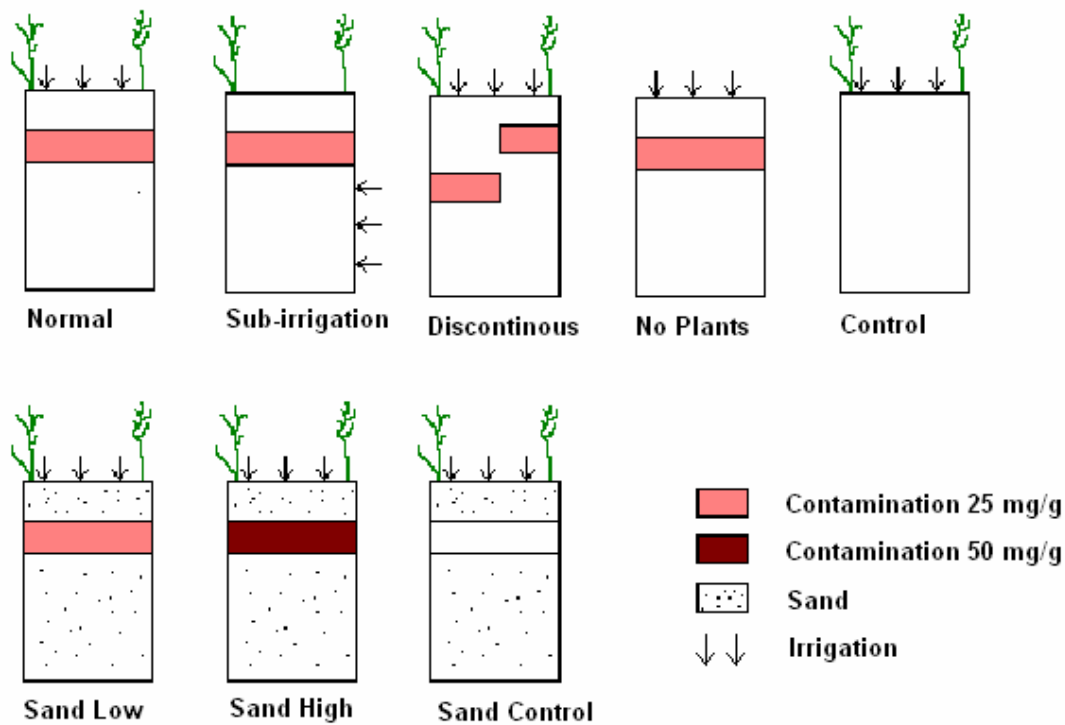


Figure 3.1 Two set of experiments, one with five treatments and one with three.

The second experiment (Figure 3.1 Two set of experiments, one with five treatments and one with three.) had three treatments replicated only once due to time limitations. All the treatments in the second experiment had a layer of sandy loam in an otherwise sandy profile to look at the effect of textural differentiation and response of diesel contamination on root development and distribution. The thicknesses of the layers were 2.5 cm of sandy loam soil covered by 5 cm of uncontaminated sand. The concentrations of the sandy loam layer were 0 mg/g (*Sand Control*), 26 mg/g (*Sand Low*) and 51 mg/g (*Sand High*).

The size of the boxes was 2.5 cm thick, 25 cm wide and 35 cm high. The backside was 12 mm thick plywood and the glass front was 8 mm. The plywood was screwed on to a frame and sealed with silicon. The glass front was held in place using washers screwed on to the frame. To avoid water logging a drainage hole was drilled in the lower side of each box and a coarse sand drainage layer, approximately 2 cm thick was added at the bottom of the boxes.

The two soils used were a sandy loam from the farm at Cranfield University at Silsoe, sieved through a 2 mm sieve, and a commercial fine sand (British Standards (BS-4500) Fraction E (particle size ranging from 90 to 150 μm)). The sandy loam was packed, at a moisture content of 7 %, in the rhizoboxes. The packing was done in 1.5 cm layers and slightly compacted to reach a final dry bulk density of approximately 1.3 g/cm^3 . Before the layer of contaminated soil was added the profile was saturated and allowed to drain.

The sand was poured into the rhizoboxes air-dry and no packing was required.

Diesel was obtained from the local petrol station and mixed with air dry soil (1 % moisture content) on a mass basis. The soil was mixed in 200 g portions together with 5 g of diesel in a glass beaker using a glass rod and stirred thoroughly and let to rest over the night.

Twelve *Lolium perenne* (Perennial ryegrass) grass seeds were seeded in three discrete locations in each rhizobox and after germination the three healthiest looking grasses were left and the rest uprooted. Locations with less than three plants had the quota filled by transplanting plants germinated in compost. The plants in the Sand experiment were all transplanted in a small compost capsule 11 days after seeding in compost (Table 3.1). The *L. perenne* was chosen because of its proven capacity to remediate diesel (Hou et al., 2001, Kaimi et al., 2004), its fibrous root system, its rapid establishment and growth (Barenbrug Homepage) and the fact that it is commercially available. The seeds used came from Barenbrug.

During the germination and establishment phase the boxes were irrigated every day. After three weeks, an irrigation scheme was set up where all treatments received 120 ml of water every third day. Due to varying temperatures in the greenhouse, ranging from approximately 10 – 45 °C, the water requirement varied accordingly and additional irrigation was supplied in terms of need. All boxes were subjected to the same amount of irrigation at all times.

To minimize any effect of the location of the boxes within the greenhouse both the location and the boxes with various treatments were numbered from 1-18 and then completely randomized. The boxes were placed on a rack in a greenhouse at an angle of 35 ° to force the roots to grow along the glass front to allow visual observations and the glass front covered with black plastic Figure 3.2.



Figure 3.2 The experimental setup with 18 rhizoboxes tilted 35° and covered in black plastic.

The angle at which the rhizobox is tilted varies in the literature. Sandnes (2005) inclined the rhizoboxes 40° and Schwartz et al. (1999) 45°. With a greater angle the natural growth pattern of the roots is more affected as the normal growth pattern is disrupted and controlled. As the contaminated layer in this study was placed close to the surface of the profile it was of importance that the roots reached the glass front before they reached the contamination. The minimum angle for this to happen was calculated according to Figure 3.3.

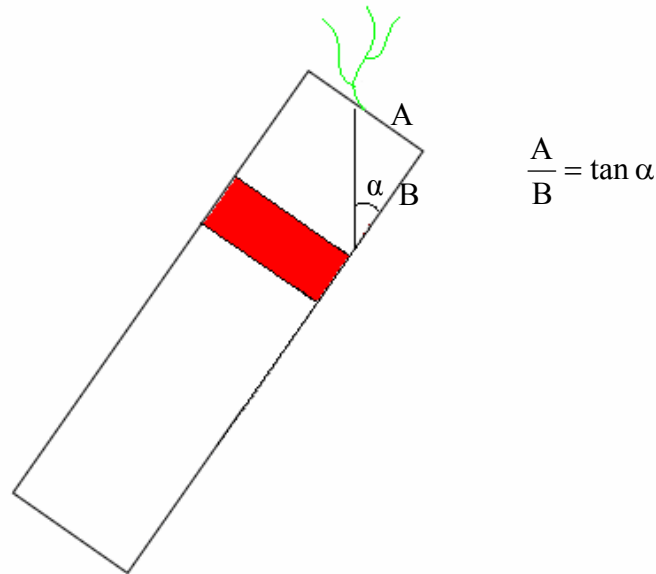


Figure 3.3 A schematic picture of a rhizobox tilted to the angle $\alpha = 35^\circ$ for the roots to reach the glass before reaching the contaminated layer.

3.2. Germination experiment

Two germination experiments were carried out. One was to investigate the viability of the *L. perenne* seeds used which was done in small pots with compost irrigated from the bottom. To examine the effect on seed germination by the diesel concentrations used in the study, a second germination experiment was conducted in the same sandy loam used in the rhizoboxes. The concentrations were 26 mg/g, 51 mg/g and an uncontaminated control. Both experiments were placed in the greenhouse.

3.3. Monitoring plant development

Root development was recorded by tracing them on OH-paper with a thin waterproof pen. The paper was then photo copied with a 30 % reduction in size (A3 to A4) and scanned at 600dpi to obtain digitised images. The subsequent recording was then made on the same paper continuing by adding the roots that developed from the last tracing. To ensure that the paper was placed in the exact same position each time matching markings were made on the glass and paper. All visible roots were recorded but differences in root diameter could not be distinguished on tracing paper. In the case of very dense roots systems, some small lateral roots might have been overlooked. The recordings were done every third day in the beginning of the recording period when root development was very rapid and every fourth day after that. The time from the first to the last recording was 27 days. The time of drawings in relation to seeding, is shown in Table 3.1 below.

To transform the root drawings to a non-graphical form, the image analysing freeware ImageJ was used. The software counts the number of pixels in each drawing originating from roots. By dividing the total number of pixels by the average thickness of the pen, a total length of

roots, in pixels, was obtained. This root length could then be converted to the metric system by using a scale previously drawn on the OH-paper used for tracing the roots.

Table 3.1 Time frame for the experiment with time in days from seeding to terminating the project

Time, t	Action
-23	Seeding
-15	Seeding in compost
0	Transplanting seeds from compost to sand experiment
1	First root drawing
4	Second root drawing
7	Third root drawing
11	Forth root drawing
15	Fifth root drawing
20	Sixth root drawing
23	Seventh root drawing
27	Eighth root drawing
28	Termination

Shoot development was measured by identifying each individual plant and record the maximum height and number of shoots, every third day. When doing so, only living and green parts of the shoots were considered in which case it was possible to have a reduction in both shoot number and shoot height. Height was measured with a ruler using the soil surface as a datum.

When the experiment was terminated the plants were harvested and both root and shoot biomass was measured. All surviving plants at each location (three per box) were removed from the soil and washed with water and oven dried in 102 °C for 18 h.

3.4. Total Petroleum Hydrocarbon Measurements

To measure the dissipation rate of diesel in the soil during the experimental period, an FTIR spectrometer, Equinox 55 from Bruker, was used. It measures the total petroleum hydrocarbon (TPH) content in the soil. Before using the method it needed to be calibrated, creating a calibration curve using soil of known diesel concentrations.

3.4.1. Calibration

To account for the variation in the soil and to create a valid calibration curve (Figure 3.4) for TPH measurements, soil from three slightly different locations, but still the same soil as used in the experiment, was used to create a series of soils with increasing diesel concentrations. To remove the diesel from the soil matrix into a liquid phase 0.5 g of wet soil was shaken for 2 min with 20 ml of acetone and 0.1 g of MgSO₄ which was added to absorb any water in the sample. A number of small glass beads were also added to break up any

aggregates in the sample. The solution was then filtered through Whatman filter paper number 114 into a glass bottle and sealed with a glass stopper to avoid evaporation. 50 µl of solution was then extracted using an automatic pipette and placed on the lens and left for 2 min before scanning to let the acetone evaporate, leaving only the diesel. The program OPUS was used to interpret the data. Before each sample scan the lens was cleaned with acetone and dried with a paper tissue three times and a background scan was performed.

To create a calibration curve, the obtained spectrum was integrated and plotted against the amount of diesel in the analysed sample ($\text{TPH}_{\text{sample}}$) (

Figure 3.4). From preparing the soil samples, the concentration, C , was known (mg diesel / g dry soil), as was the dry weight, W_D , of the sample. The total amount of TPH in the sample could then be calculated accordingly:

$$\text{TPH}_{\text{sample}} = C \cdot W_D$$

When analysing samples with unknown concentrations the $\text{TPH}_{\text{sample}}$ was obtained from the calibration curve and the concentration thus gained by dividing $\text{TPH}_{\text{sample}}$ with the dry weight of the sample.

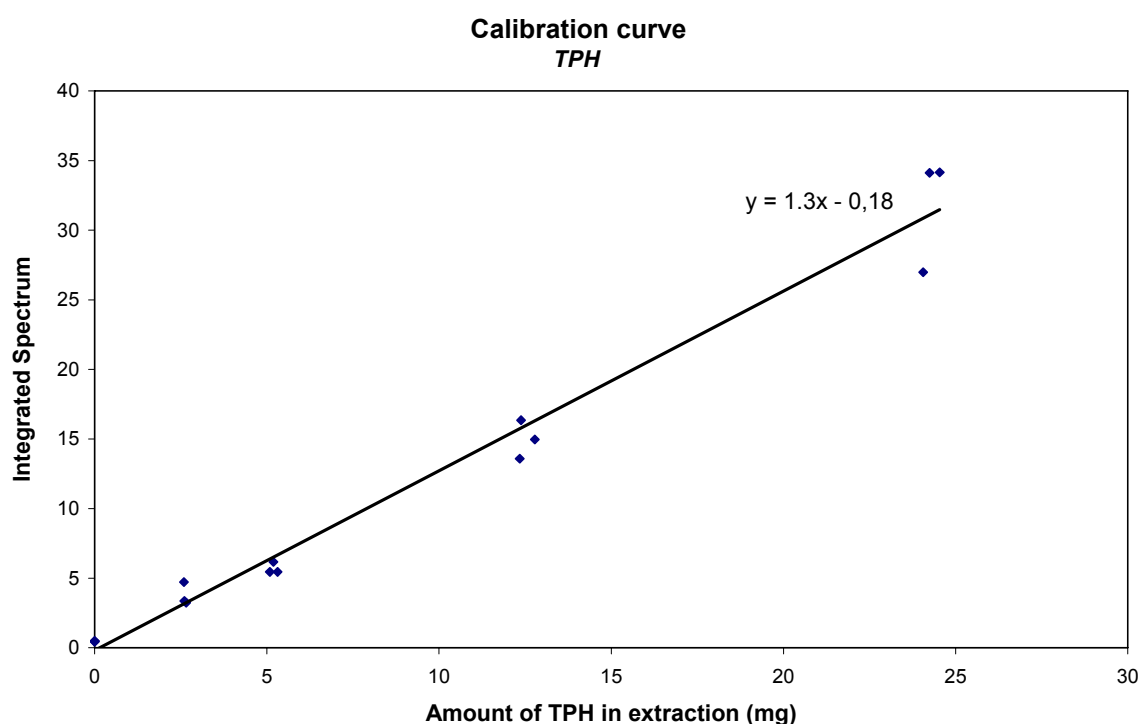


Figure 3.4 Calibration curves for measuring TPH by integrating the TPH spectrum (no unit) with the equations displayed in the diagram. The r^2 value for the calibration curve is 0.98.

3.4.2. Soil sample analysis

When the experiment was terminated the rhizoboxes were dissembled and the soil analysed. Seven soil samples per box were analysed for TPH content; two from the layer above (Surface Layer) and two from the layer below (Sub-layer) the contamination to investigate any possible movement of diesel, either upwards or downwards, in the profile. Three samples were taken from the contaminated layer to investigate the dissipation rate of the diesel. For the control without contamination a total of three samples per box were analysed since there was no layering.

The calibration curve described above was created on a dry soil basis. To take into account the total liquid content (water + diesel) in the samples to be analysed, two soil samples from each layer were oven dried for 48 h and the liquid content calculated. The method of analysing was the same as described in the calibration method. The difference was that 1.5 g of soil was used instead of 0.5 g together with 0.3 g of MgSO_4 .

3.5. Statistics

The results from the biomass, shoot and TPH measurements were analysed statistically using ANOVA. The textural experiment with sand was not replicated and could thus not be analysed.

4. RESULTS

All root drawings are displayed in appendix 2 and statistical analysis of significant differences between treatments are to be found in appendix 1.

4.1. Germination rate

The germination experiment (Table 4.1) showed that the viability of the seeds used was good but that the germination rate of *L. perenne* is severely affected by the diesel concentrations used in this study. Only 1 out of 30 seeds germinated in soil contaminated with 26 mg/g of diesel compared with 100 % germination rate in clean soil. In the soil with 51 mg/g no seeds germinated at all.

Table 4.1 Percentage germination of *L. perenne* in different soil and diesel concentration. No concentration dependent germination experiment was done in the compost (*)

	Diesel concentration (mg/g)		
Soil type	0	26	51
Compost	89	*	*
Sandy loam	100	3	0

4.2. Treatment Effect on Plant Performance

4.2.1. Layering

Adding a thin layer of fresh soil on top of a contaminated layer secured germination and initial establishment, but further plant development was severely effected by the presence of diesel. As can be seen in

Figure 4.1 and Figure 4.2, the diesel contaminated treatments show a significantly lower root and shoot development compared with the un-contaminated control.

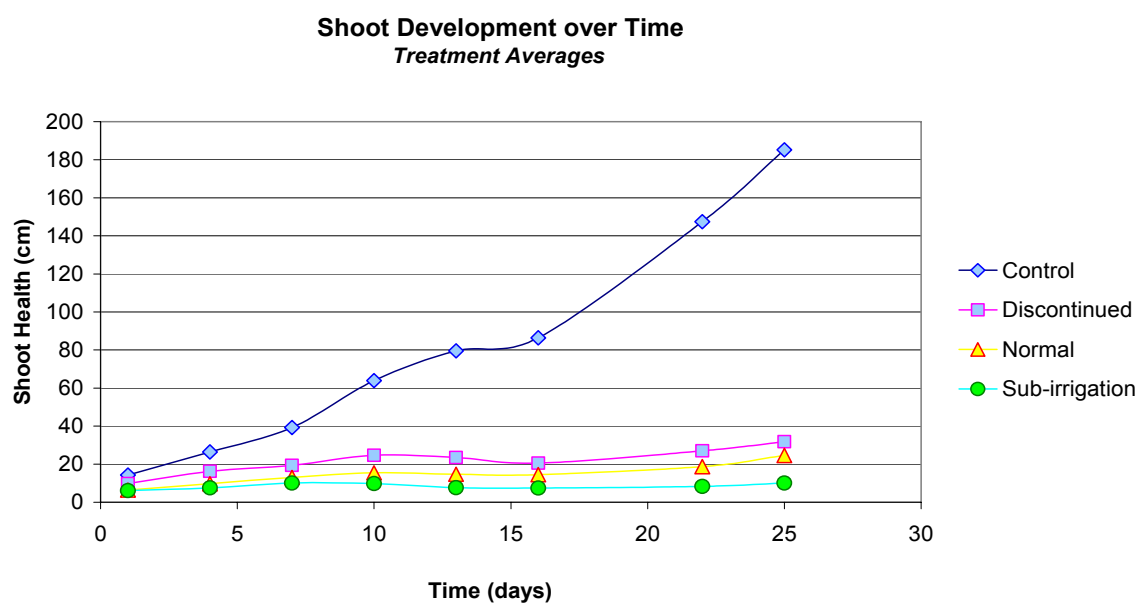


Figure 4.1 Shoot development, expressed as maximum shoot height times shoot number for each plant, over time

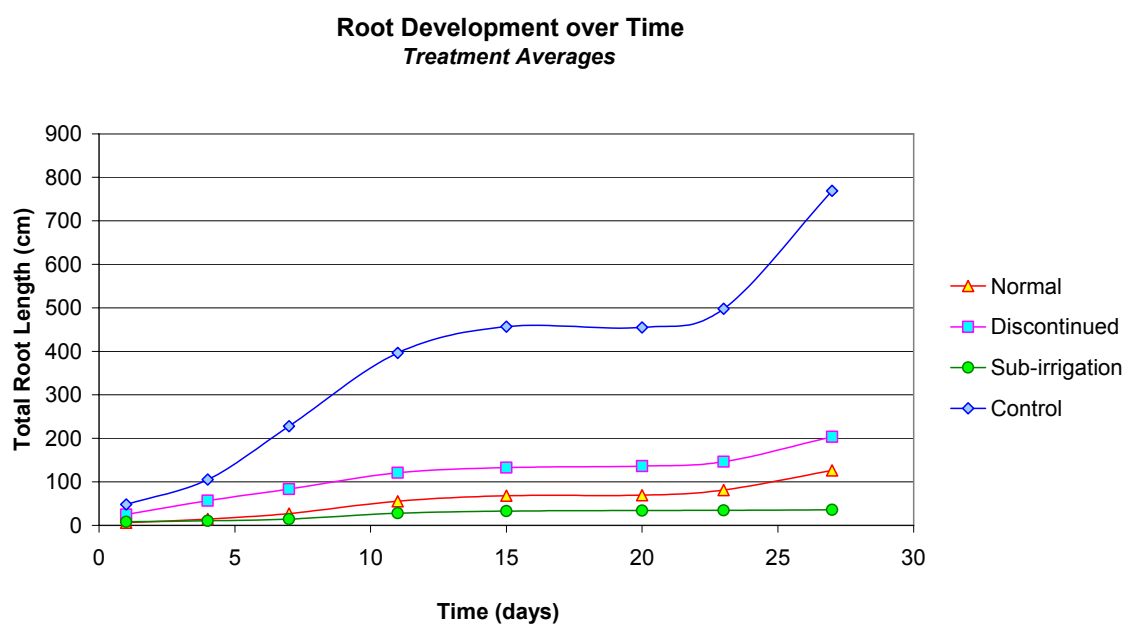


Figure 4.2 Root development over time as treatment averages, derived for root drawings.

Table 4.2 Percentage reductions in root and shoot dry biomass compared with Control

Treatment	Reduction in dry biomass (%)	
	Root (%)	Shoot (%)
Normal	81	92
Discontinuous	79	82
Sub-irrigation	99	94
Sand High	53	68
Sand Low	51	52

The root biomass at time of harvest was 81 % lower for the Normal treatment compared with the Control and shoot biomass 92 % lower (Table 4.2 and Figure 4.3). The *shoot health*, which in this study is defined as the highest shoot multiplied with the number of shoots for each plant (Figure 4.1), together with root length (Figure 4.2) show a very similar development over time with the Control having a much superior plant development than all the diesel contaminated treatments.

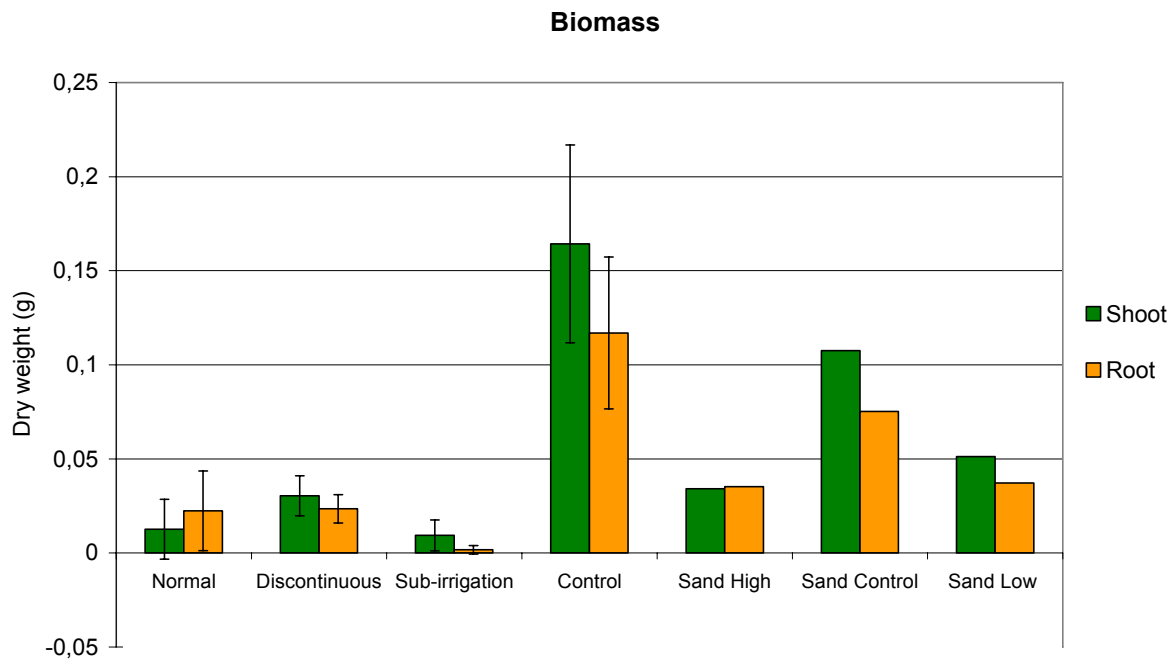


Figure 4.3 Root and shoot biomass with standard deviation as error bars. The Sand treatments were not replicated and therefore have no error bars.

The root behaviour when coming across the contaminated layer is depicted in Figure 4.4. In one of the replicates (Box 10) the layer functions as a barrier that the roots only partially penetrate. Once they have grown through there is more spreading below the contaminant than in it until $t = 27$ days where the roots start to spread laterally in the layer. One of the other replicates (Box 1) showed tendencies towards the same distribution behaviour but not as clearly. Box 6, of the Normal treatment (see appendix 2) had a very low plant performance with plants withering and dying and therefore not showing any root development.

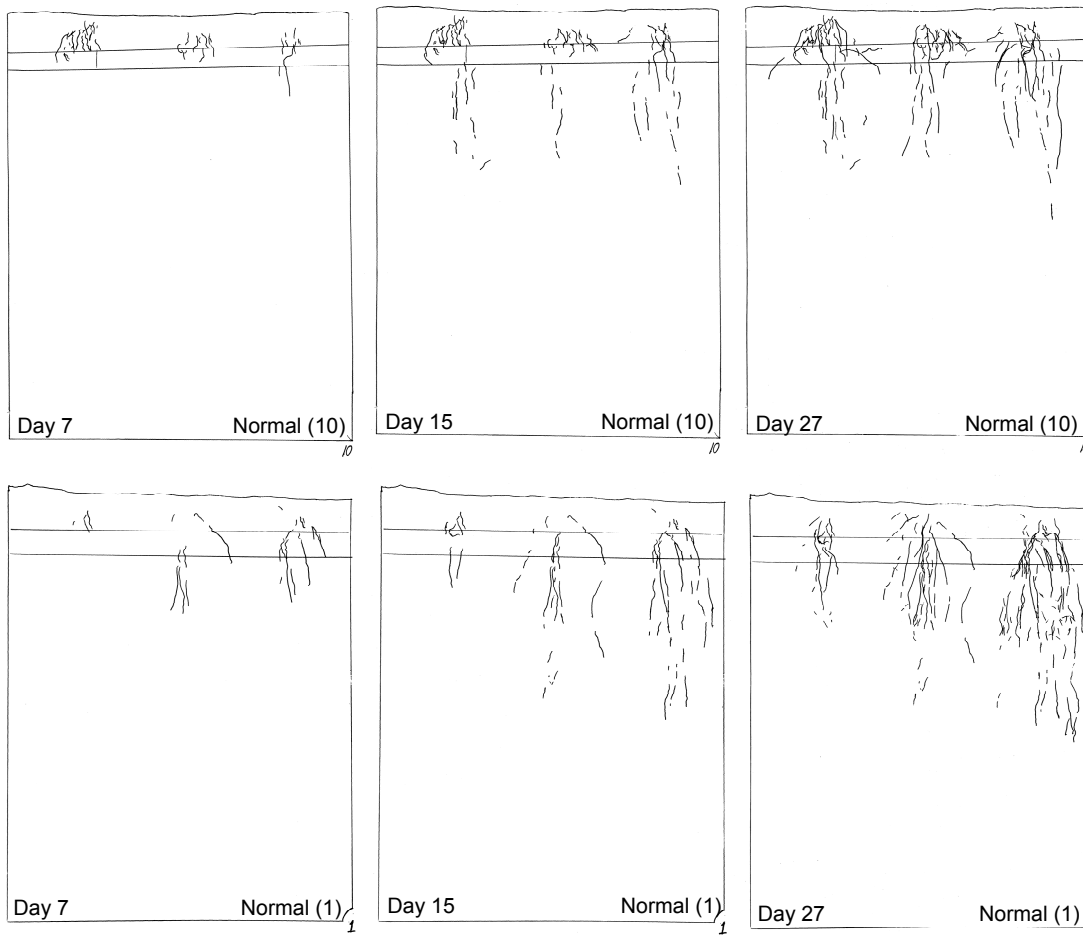


Figure 4.4 Two of the three replicates of the Normal treatment, boxes 10 (upper row) and 1 (lower row). In box 10 the roots are not growing into the contaminated layer until $t = 27$ days whereas in box 1 the response in root distribution by the presence of a contaminated layer is less evident.

In the case of Discontinuous layering the effect of the thickness of the uncontaminated soil covering the contaminated layer, is clear (Figure 4.5). Where the roots have a thicker layer of uncontaminated soil to spread in, they do not penetrate the contaminated soil until $t = 27$ days. This phenomenon occurs in all three replicates, Boxes 2, 4 and 15. Where the contamination is disrupted, a passage of clean soil from the surface to the clean soil below the contamination is created. The roots of the plants grown just above the fault show the same distribution pattern as plants grown on the thick layer of fresh soil, mainly spreading in the thick surface soil. The roots do not show any particular increase in density in the passage of clean soil.

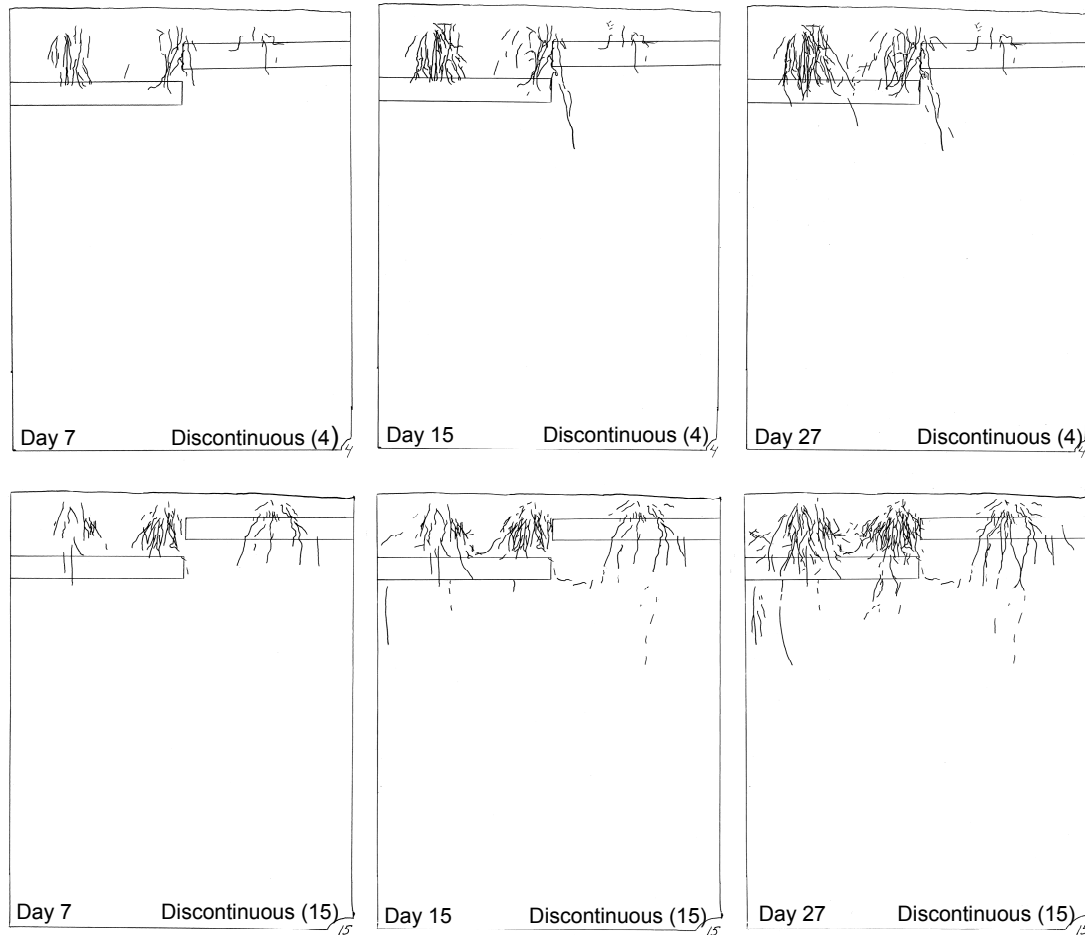


Figure 4.5 Two different replicates of the Discontinuous treatment, boxes 4 (upper row) and 15 (lower row). Roots clearly not growing into the contaminated layer. Box 2 can be viewed in appendix 2.

A thicker layer of clean soil above the contaminated soil has a beneficial effect on plant performance. The Discontinuous treatment has the most successful plant development of the diesel contaminated treatments with highest survival rate (Figure 4.6) and significantly ($p = 0.05$) longer total roots, higher biomass, and shoot health, over time compared to both the Normal and the Sub-irrigated treatments (Figure 4.1 - Figure 4.3 and tables A1 – A14 in appendix).

4.3. Sub-irrigation

Sub-irrigation clearly had a negative effect on plant performance. During the period of the experiment, 60 % of the plants died (Figure 4.6) and the surviving ones showed severe stress symptoms like change in colour and dwarfed growth compared to the Control. There was only a minimal root and shoot development; the root biomass was 99 % lower than the control and shoot biomass 94 % lower (

Table 4.2). Before the start of the irrigation scheme the Normal and Sub-irrigated treatments were both irrigated from the top for three weeks, from seeding until the irrigation treatment and measuring began at $t = 1$ day. Up to that point and until $t = 7$ days they both showed the same plant development which is apparent as the two treatments follow the same root and shoot health development curves for that time frame (

Figure 4.1 and Figure 4.2). After a week the two treatments start to deviate; the Normal treatment shows a slight development whereas the Sub-irrigated treatment shows no increase in root and shoot development.

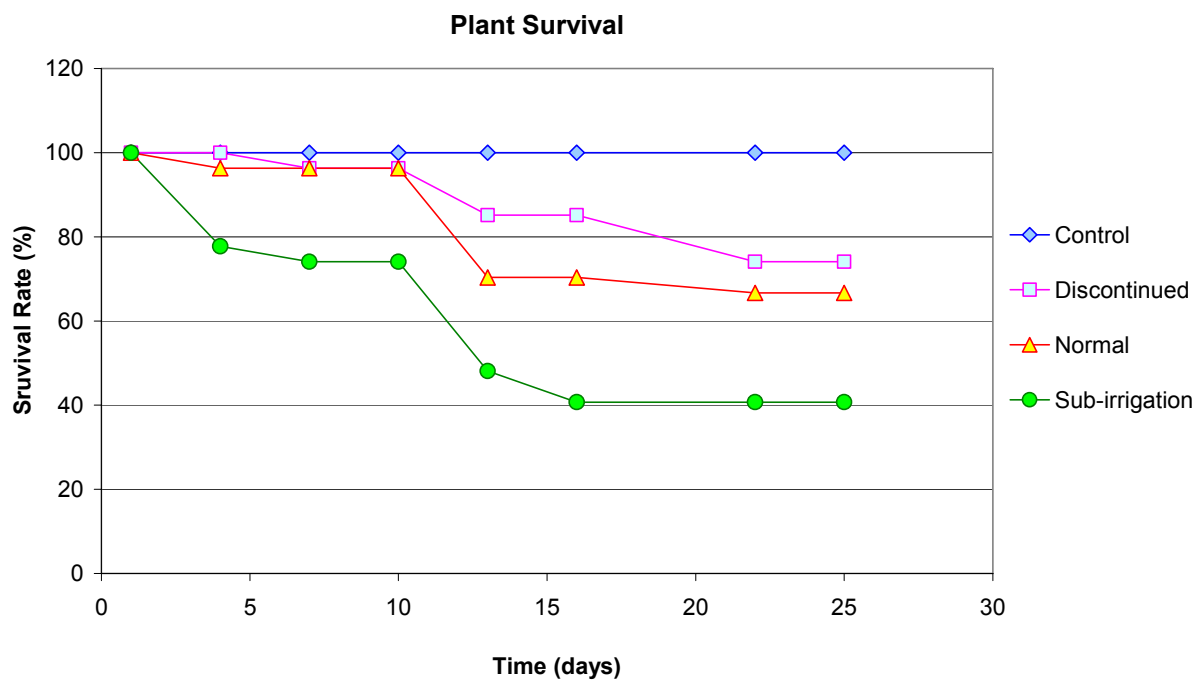


Figure 4.6 Survival rate in percentage from time $t = 0$ for different treatments.

4.3.1. Textural differences

A coarser soil texture above a finer layer of contaminated soil had an effect on the spatial distribution of the roots. When comparing the controls of the experiment with texture differentiation (Sand Control) and the Controls in the homogenous soil experiment they display very different growth patterns (Figure 4.7). The roots of the Control in the homogenous soil have a distinct downwards growth, with the roots reaching a maximum of 30 cm at the end of the experiment period (Box 9) compared with 14 cm for Sand Control (Box 13). The root drawing (Figure 4.7) illustrate that the roots tend to spread and become more dense in the finer textured layer and that further downward growth is not occurring to any great extent.

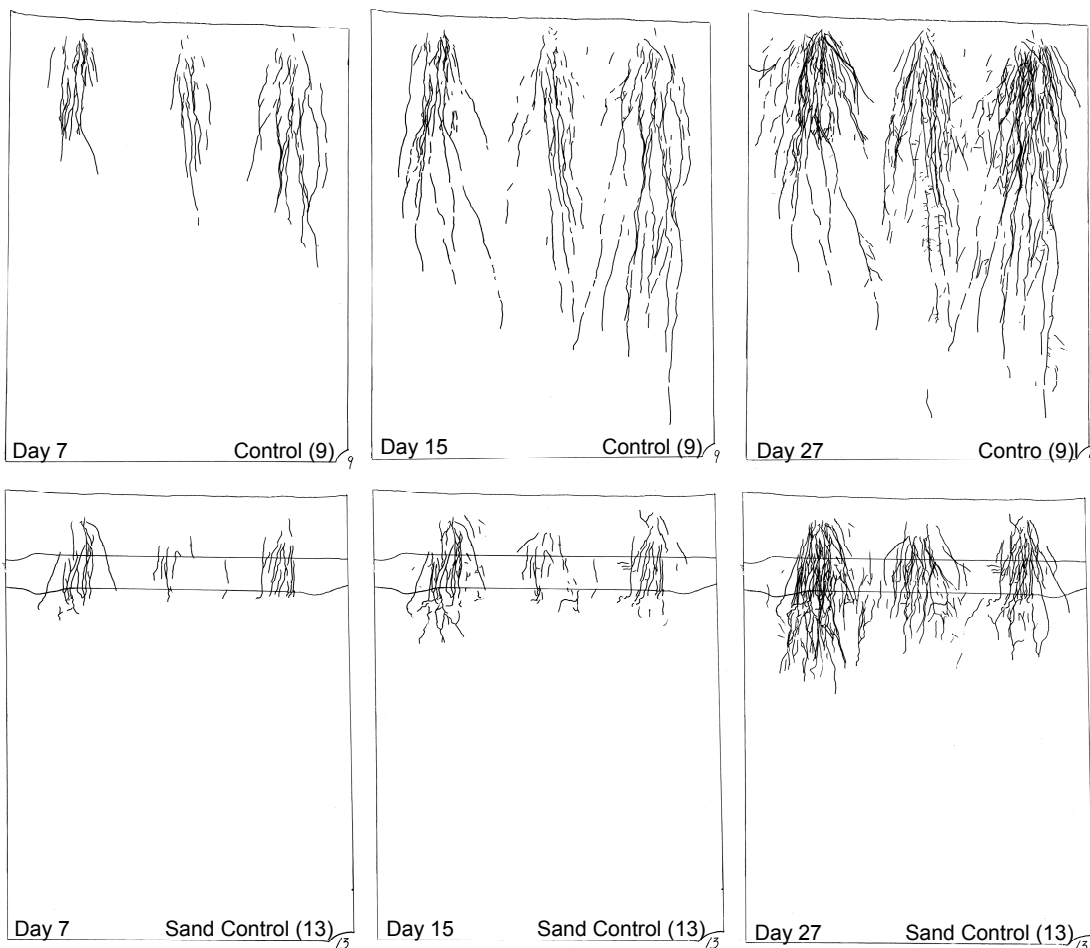


Figure 4.7 The top set of drawings one of the Controls from the experiment with uncontaminated sandy loam throughout the profile. The set below is the Sand Control from the textural experiment with a layer of uncontaminated sandy loam in an otherwise sand profile.

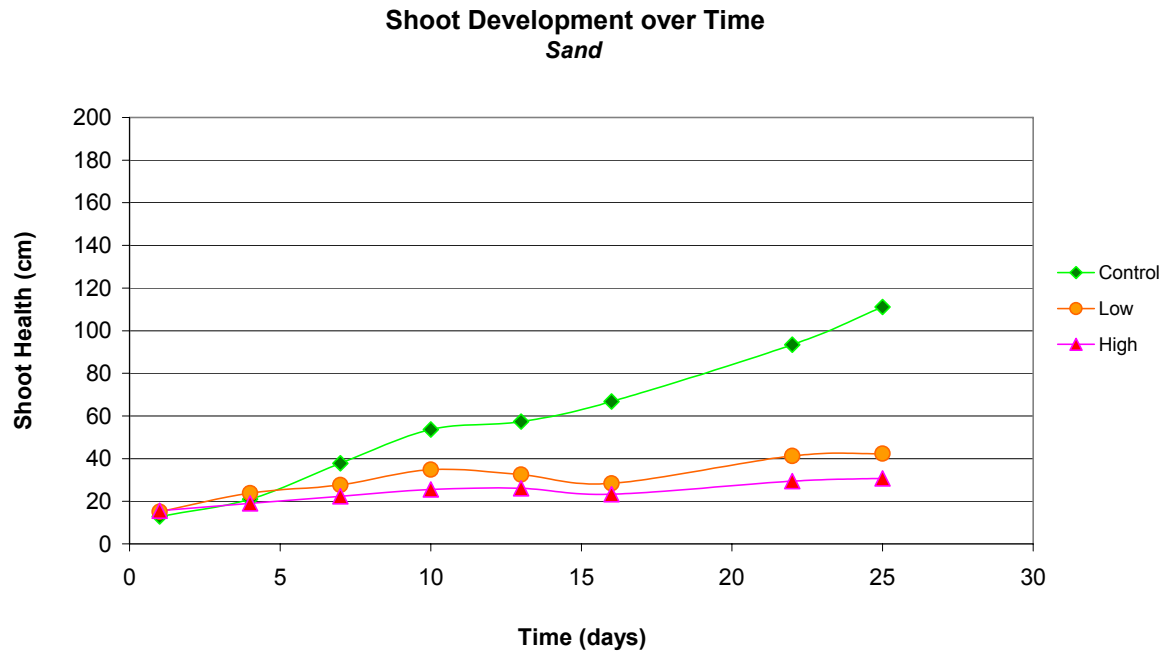


Figure 4.8 Shoot development, in terms of Shoot Health (number of shoots times shoot height) over time in the textural experiment with layering of different soil textures

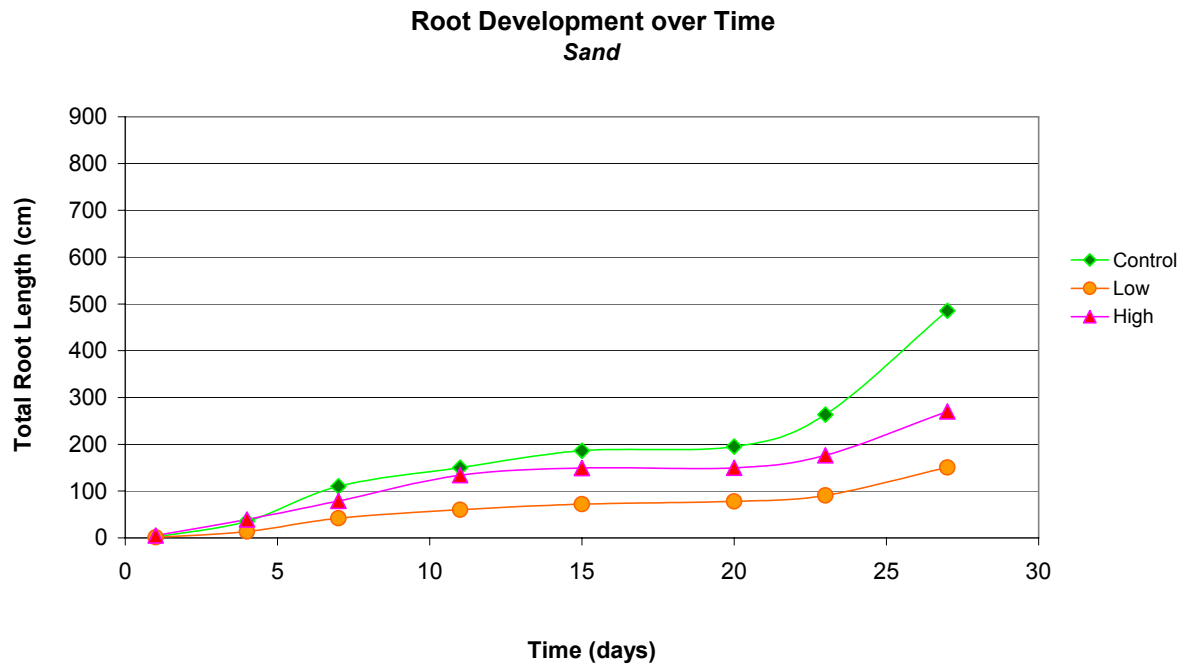


Figure 4.9 Root development in textural experiment over time, derived from root drawings.

The effect of different diesel concentrations is not conclusive. Where the diesel concentration was 50 mg/g (Sand High), the root development is significantly *higher* than when the concentration was only 25 mg/g (Sand Low) as can be seen in Figure 4.9 whereas the shoot development is significantly lower (Figure 4.8). Compared with the control of the sand experiment (Box 13, Figure 4.7), both the treatment with high concentration (Box 11, figure 5.10) and the treatment with low concentration (Box 17, Figure 4.10) have significantly lower root length. The root drawings of the treatment with Low concentration show that the roots do not grow into the contaminated layer until $t = 27$ days in accordance with the Normal and Discontinuous treatments in the experiment with homogenous soil. The treatment with High concentration shows no such tendency and the roots seem to even spread in the finer textured contaminated layer in a similar way to the control in the sand experiment.

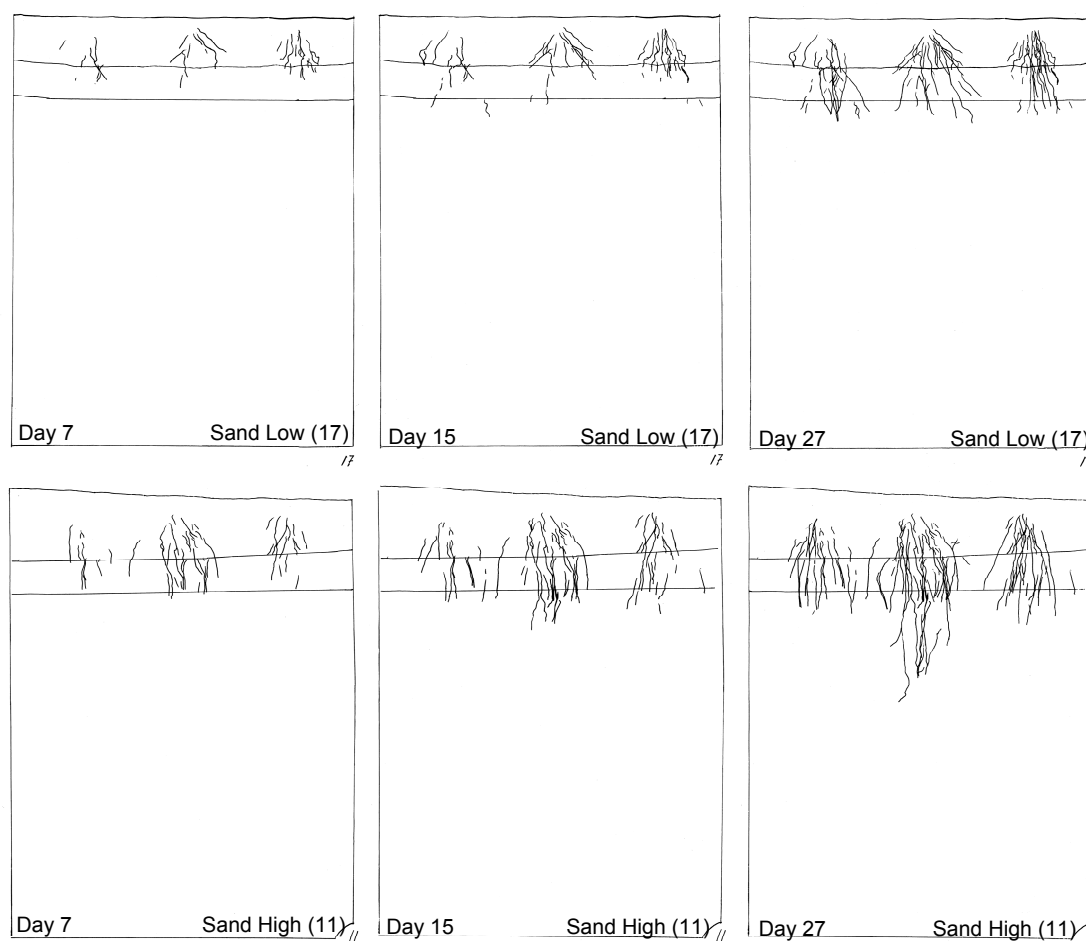


Figure 4.10 Comparison of root development between Sand Low (upper row, box 17) (25 mg/g) and Sand High (lower row, box 11) (50 mg/g) in experiment with textural differentiation with a layer of sandy loam embedded in sand.

4.4. Treatment effect on TPH dissipation

The results of the TPH analysis show that there are only small differences in the breakdown effectiveness of the diesel between the different treatments. For all replicated treatments in the homogenous soil experiment, TPH dissipation of the contaminated layer is between 81 % and 84 % which is illustrated in Figure 4.11. The control treatment with No Plants shows the lowest dissipation rate together with the Sub-irrigated treatment, both having a reduction of 81 %. This is significantly lower ($p = 0.05$) than the dissipation in the Normal treatment but not compared with the Discontinuous treatment (table A14 in appendix 1). There is no difference in TPH dissipation rate between the Normal and Discontinuous treatments and between the treatments with Sub-irrigation and No Plants. The TPH dissipation rate for the sand experiment with a contamination level of 50 mg/g of diesel (Sand High) has a higher dissipation rate than the treatment with 25 mg/g (Sand Low), 82 % and 78 % respectively (Figure 4.11).

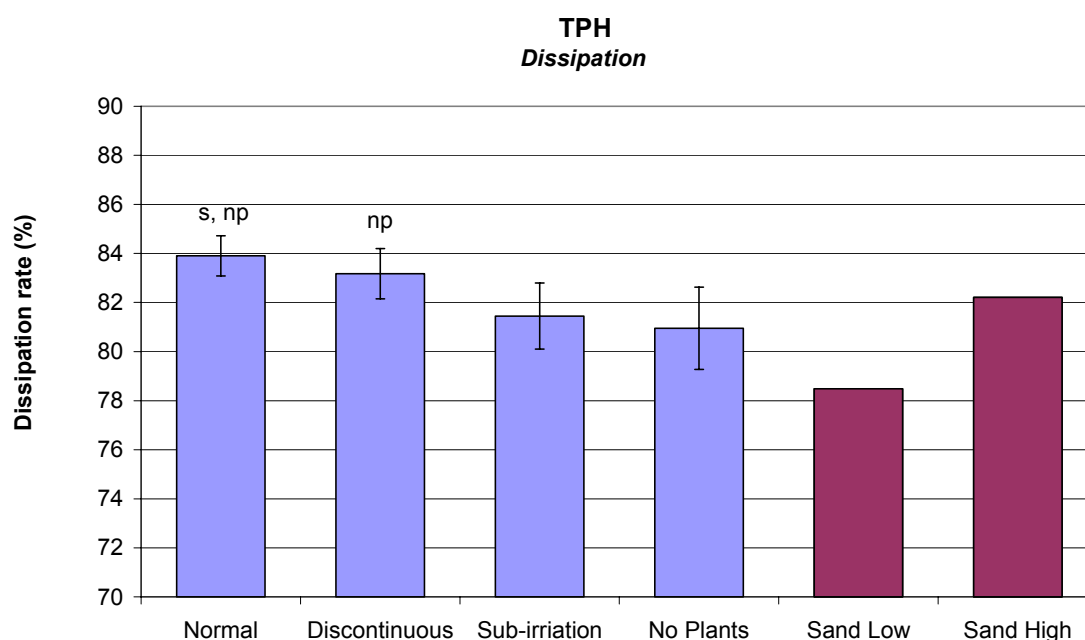


Figure 4.11 Dissipation rate for both the replicated experiment with the standard deviation as error bars and the sand experiment without statistical analysis. The labels s and np indicate significant difference from Sub-irrigation and No Plants respectively. Note that the scale is broken.

No movement of diesel either up or down the profile could be detected (Figure 4.12). The results from the TPH analysis of the surface and sub-layer did not deviate much from the uncontaminated Control, but there is a weak tendency for the treatments irrigated from above to show higher TPH concentrations in the sub-layer and for the Sub-irrigated treatment to show higher concentrations in the surface layer. However, where significant differences were observed between treatments, they were not significantly different compared to the uncontaminated control and are therefore inconclusive. The very high standard deviations in Figure 4.12 are a result of negative values of TPH concentrations from the analysis. Diesel movements could not be detected in the Sand experiments either (Figure 4.13).

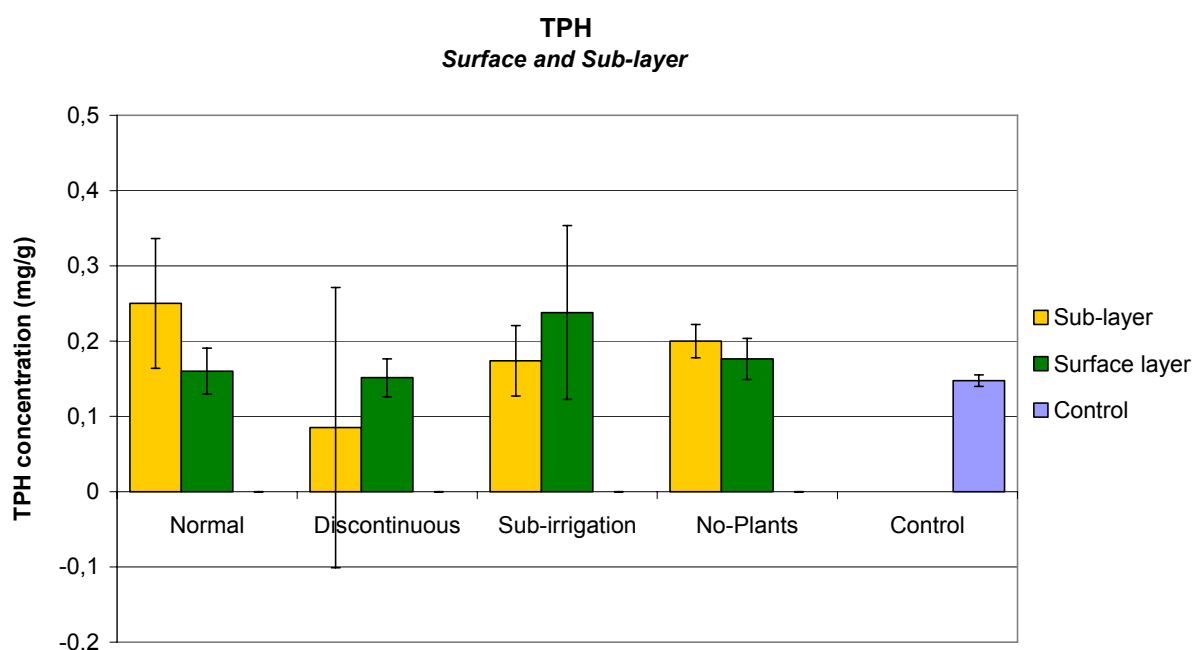


Figure 4.12 TPH concentration readings above and below the contaminated layer. The extreme standard deviation in Discontinuous is caused by two negative readings.

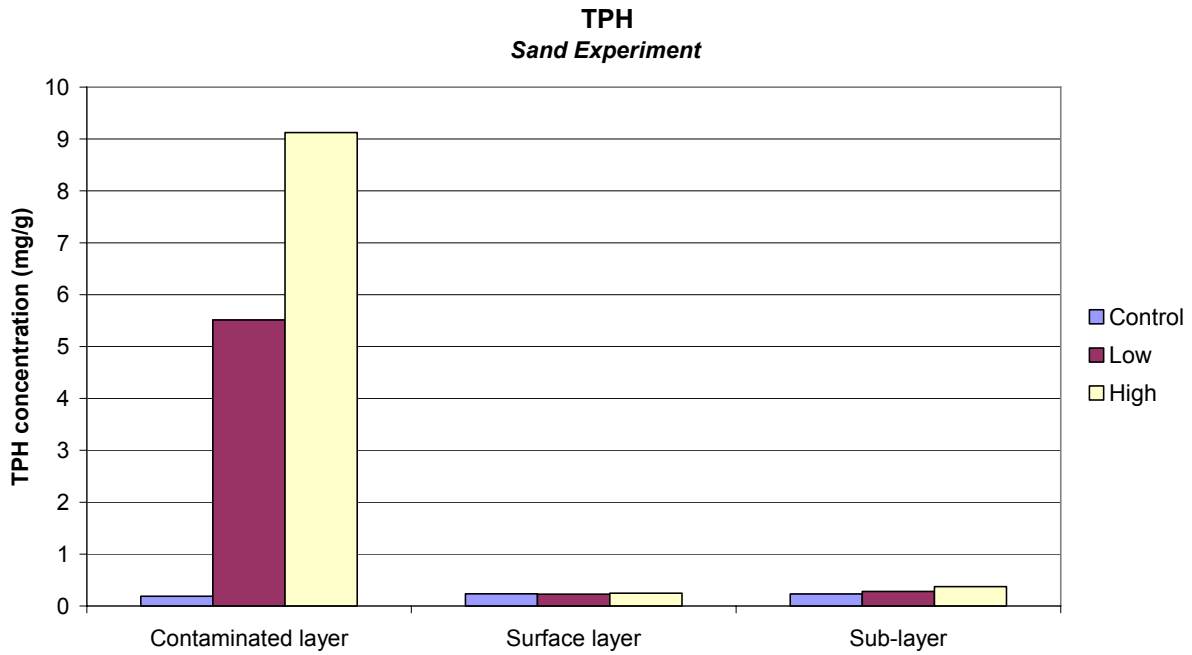


Figure 4.13 TPH concentrations in textural experiment. No significant differences in TPH concentration between treatments for both surface and sub-layer, compared with the control.

The experimental results from this study show a linear relationship ($r^2 = 0.52$) between root length and TPH dissipation which can be seen in Figure 4.14. The treatments with highest root development (Normal and Discontinuous) also show a higher level of dissipation of TPH. With a larger set of data points the relation might not be linear. The small variations in both root length and TPH dissipation only give a limited view of the relationship. The correlation curve for TPH dissipation and root *biomass* shows a weaker relationship ($r^2 = 0.33$) (Figure 4.15).

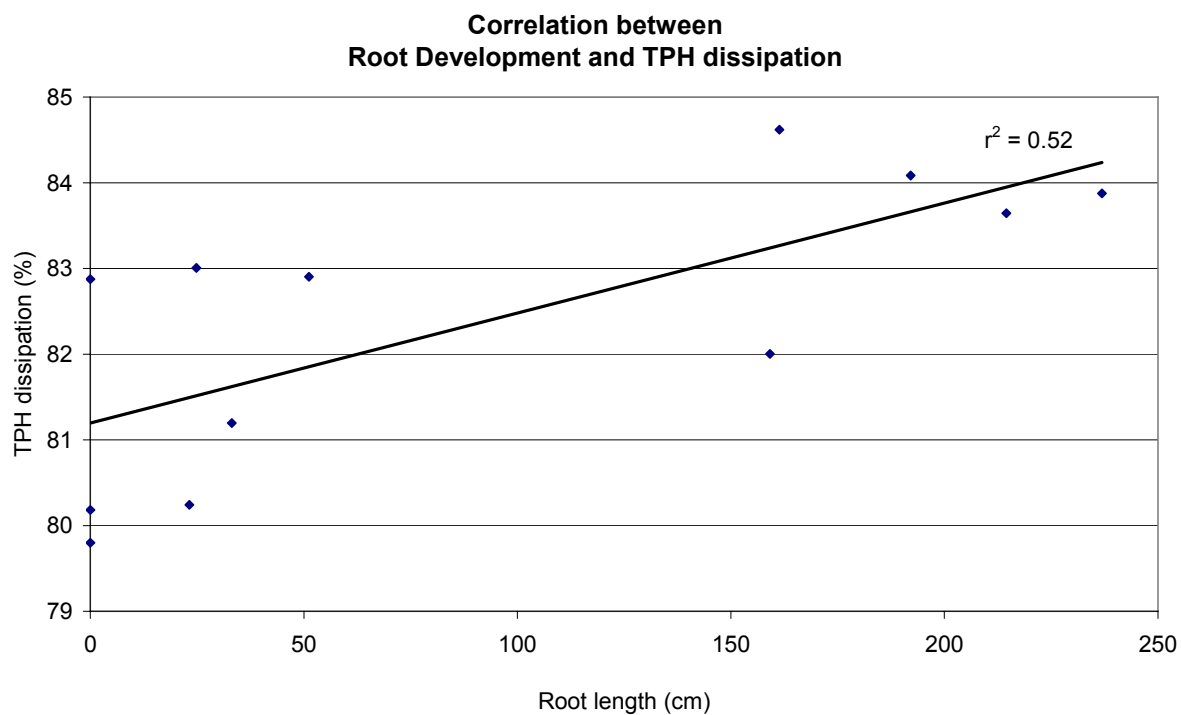


Figure 4.14 A linear relationship between root development in terms of root length and TPH dissipation. With a larger series of data points there might be another relationship.

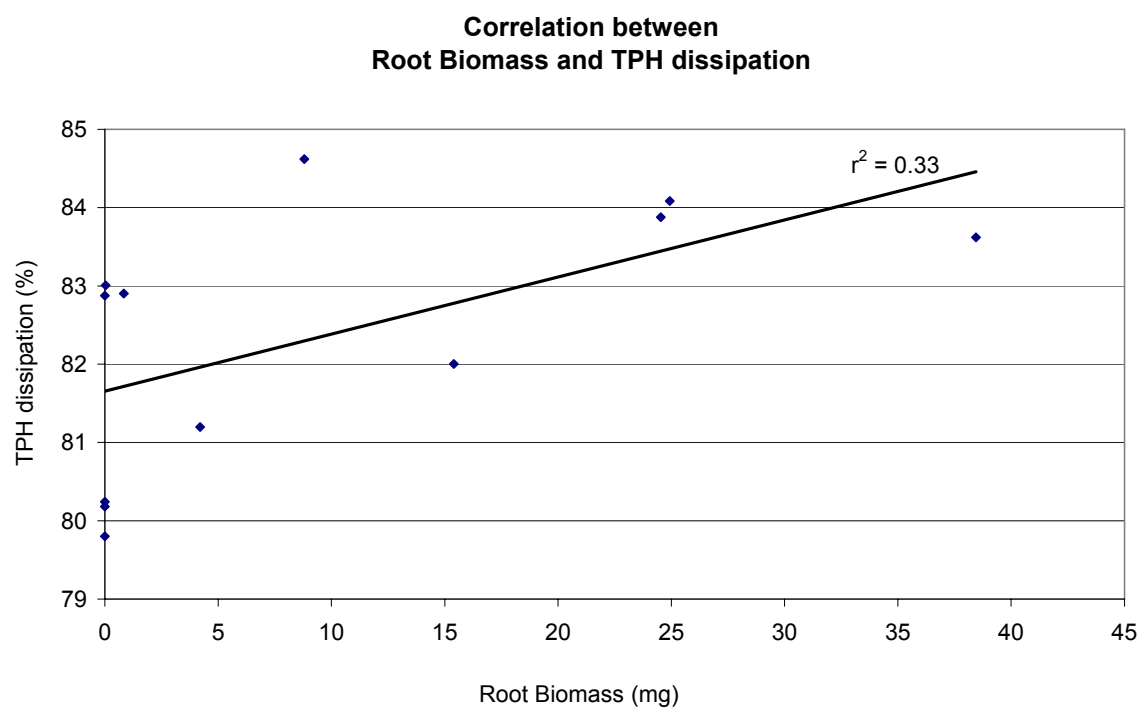


Figure 4.15 There is a weak correlation between *root biomass* and TPH dissipation.

5. DISCUSSION

5.1. Effect of root development on diesel dissipation rate

Because of the limited time of this study, a strong correlation between root growth and diesel dissipation was not expected. The correlation coefficient of $r^2 = 0.52$ indicate that there is a relationship, but the snapshot nature of this study only gives a very partial picture of the full extent of the relationship. A number of studies show a clear correlation between root growth and TPH dissipation (Kaimi et al.; 2004, Merkl et al., 2005) but that the positive influence does not become apparent until the roots have fully developed, which for *L. perenne* is about three months (Hou et al., 2002). This is three times longer than the extent of the growth period in this study. Hou (2002) showed that during the first month all treatments deviate very little from the dissipation curve of natural attenuation (No Plants). With decreasing levels of diesel the dissipation rate for natural attenuation start to level off but the breakdown continues in soil influenced by roots. In this study, it is likely that a stronger correlation between root length and TPH dissipation would have formed if the experiment had been run for a longer period of time.

Although the overall correlation between root development and TPH dissipation is not very strong there are still some tendencies worth noticing. The two treatments that had the lowest, or no root development at all, Sub-irrigation and No Plants, also had the lowest dissipation rate and the Discontinuous and Normal treatment which had the highest root development also had the highest dissipation rate. In the Sand experiment the effect of root density on TPH dissipation is also apparent. The significantly higher root length in the treatment with High diesel concentration correlates with a significantly higher dissipation rate compared with the treatment with Low concentration.

5.2. Effect of layering on root behaviour

The negative influence on plant development by the presence of diesel has been documented in several studies (Hou et al., 2000; Palmroth et al., 2002). It is clearly shown in this study that diesel contamination has a negative effect on plant and root growth with statistically significant differences in root and shoot biomass, root length, the height and number of shoots, between the treatments containing diesel and the controls. For example the worst affected treatment shows as much as 99% lower root biomass than the control.

For roots to have any positive impact on diesel dissipation rate, they need to be present inside the contamination where they will enhance the microbial activity which is what provides the increase in hydrocarbon breakdown (Kaimi et al., 2004). The thicker layer of clean soil above the contaminated layer in the Discontinuous treatment, helped the plants to establish and develop without the roots having to penetrate the diesel contaminated soil. The Roots in the Normal treatment, which had a thinner layer of clean soil to grow in, were forced to seek moisture further down since the thin surface layer did not provide enough moisture to sustain growth. The fact that they had to grow through the contaminated layer caused a stronger toxic response compared with the Discontinuous treatment and thus showed a lower plant development. When comparing the two treatments in terms of TPH dissipation however, the Normal treatment with lower root length showed a slightly higher level of dissipation and the Discontinuous treatment with higher root length showed a lower TPH dissipation. The

difference in TPH dissipation between the two treatments was however not significant. The tendency is likely to be caused by a difference in spatial distribution of the roots with the roots of the Discontinuous treatment mostly distributed in the uncontaminated surface layer and the roots of the Normal treatment actually penetrating the contaminated soil. With time and decreasing diesel concentration, the roots of the Discontinuous treatment did start to penetrate the contaminated layer indicating that with time there might be an increase in the beneficial effect of roots on diesel degradation as the root length increases inside the contamination.

5.3. Influence of texture

The experiment with textural layering aimed at investigating the effect of moisture and diesel concentration on root development and distribution. As expected, the sandy loam layer, which had better moisture holding capacity and nutrient status than the surrounding fine sand (Hillel, 1982) was more favourable to root growth when uncontaminated, with the control having statistically higher shoot and root development compared with the contaminated treatments. The root drawings show that the roots spread in the sandy loam layer whether it is contaminated or not rather than growing further down the profile. This emphasizes the importance of good soil moisture and nutrient status on plant growth and root development in diesel contaminated soils.

The fact that significantly higher root density was observed in the treatment with double diesel concentration can possibly be explained by two factors.

- The toxic effect of diesel on plant growth might not be entirely concentration dependent. A study of toxic effect of different fuel oil concentrations on salt marsh species showed that there was no statistical effect on root biomass between fuel concentrations of 29 and 114 mg/g dry soil, even though the overall trend was that with increasing concentration there was a significant decrease in root biomass (Lin et al., 2002). In the current study only two different diesel concentrations were used. With more treatments of a larger range of diesel concentrations, it is possible that a concentration dependent response on root growth would have been able to show in this study.
- It is possible that the effect of the small compost capsule used when transplanting the seedlings into the sand was unequal between the two treatments causing unequal growth conditions. The transplanting was done by two different people and the human error should be taken into account.

5.4. Effect of irrigation on plant development

Sub-irrigation has been shown to significantly increase TPH degradation by stimulating root growth at depth (Hutchinson et al., 2001). The aim of this study was to provide water to the plants through capillary rise and thus creating a moisture gradient, stimulating a deeper root growth. However, sub-irrigation proved to have a detrimental effect on the plants in this study with 60 % death rate, 99 % lower root biomass and more than 20 times less root length compared with the control. Upward movement of water is created by a difference in pressure potential between the saturated soil at the point of irrigation and the dry soil surface. This

force can be several orders of magnitude higher than the gravitational force causing capillary rise. The hydraulic conductivity through the unsaturated soil at the surface is thousands of times lower than that of the moist soil below the source of irrigation and the bulk movement of water is thus still downwards and the irrigation water drains quickly. Therefore, compared with the treatments irrigated from above, the surface layer of the Sub-irrigated treatment will always receive less water.

It was expected that the resulting water stress would force the roots to seek moisture further down the profile and grow through the contaminated layer. The results of this study show that the water stress applied to the plants in conjunction with diesel treatment was too severe, which caused plant death. With a slower irrigation timeframe (i.e. the same amount of water applied over a longer time period) a larger proportion of the water would be likely to reach the surface and help sustain plant growth. It is possible that under such conditions the plants would be healthier and be able to seek moisture further down the profile and thus have a better remediation potential, but this would have to be investigated further.

Another contributing factor to water shortage at the surface could be due to bad connection between the contaminated layer and the surrounding soil in some of the boxes. This creates a capillary barrier that would not affect downwards water movement when irrigated from above but prevent water reaching the surface when irrigated from below (Figure 5.1).

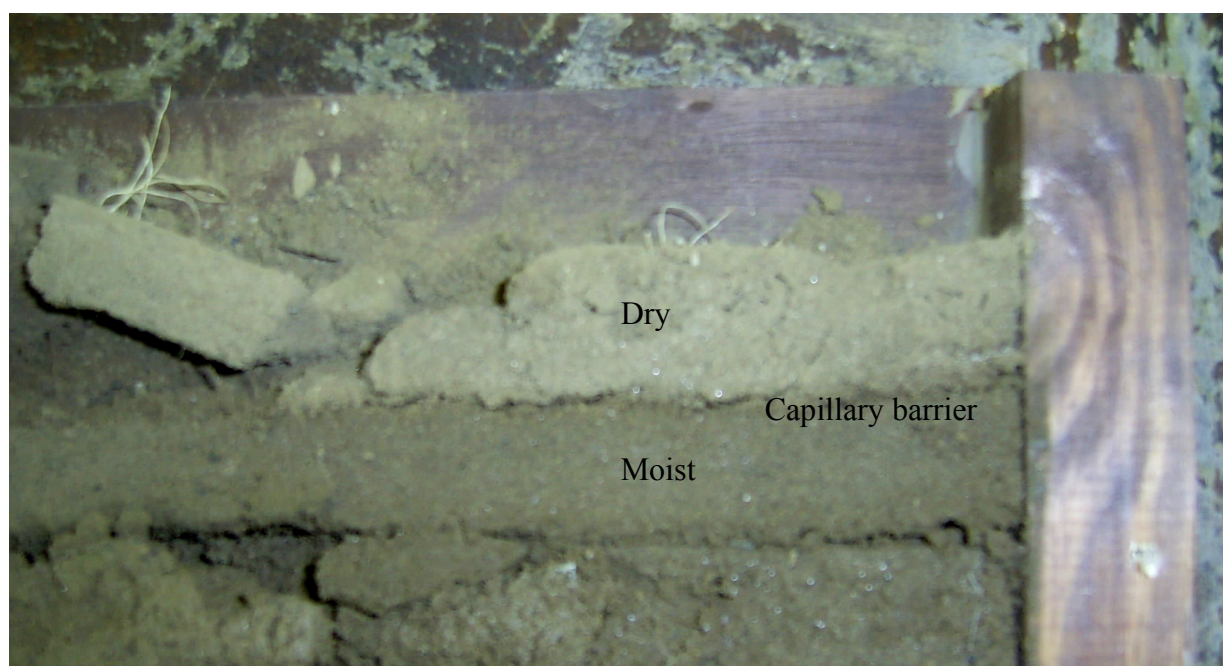


Figure 5.1 A Sub-irrigated treatment during dismantling shows a capillary barrier above the contaminated layer causing the surface layer to become extremely dry while the rest of the profile is moist.

5.5. TPH movement

For phytoremediation to be effective it is important that leaching of the contaminant does not occur beyond the reach of the roots. Leaching can also cause ground waters and streams to become contaminated increasing the risk to human health and the ecosystem. No movement of diesel could be detected in this study regardless of irrigation method. Results from the TPH analysis of soil above and below the contaminated layer deviate very little from the control. The occurrence of small amounts of TPH in the Control is more likely to be caused by inaccuracy in the calibration method rather than any actual presence of hydrocarbons in the soil because the spectrum from the FTIR spectroscopy was virtually flat. The reason this study could not show any diesel movement might have a dual explanation. A study by Adam et al. (2002) showed that free phase diesel could easily be mobilised by water down the soil profile. However in this study the diesel volumetric content was close to residual. When preparing the contaminated soil, the diesel was thoroughly mixed with the soil so that any free liquid was adsorbed to the soil particles and any organic matter in the soil. This suggests that any movement of free phase diesel is unlikely. Hutchinson et al. (2001) showed that only 0.02 % of the TPH in aged petroleum sludge was leached from the profile with irrigation water.

The lack of detectable amounts of TPH in sub- and surface layer can also be due to the fact that any displaced diesel would readily be broken down over the time of the experiment period since the dissipation by natural attenuation of the diesel was about 80 %. The spectrums obtained from the surface and sub-layer were not as flat as the control but oscillated around the zero line with a small reading at a slightly lower frequency. Each individual hydrocarbon compound has its own unique frequency and secondary metabolites can therefore appear at a different frequency. This could be what was seen in the spectrum indicating that in fact there had been a movement but that concentrations were too small to be detected with the method used.

5.6. Factors affecting experimental results

5.6.1. Limitations of the rhizobox technique

The use of rhizoboxes in this study enabled the root development to be followed visually through the glass. The predominant growth pattern of the roots is vertical through the soil and when the roots encounter the glass front they are forced to change direction to follow the angle of the box. As can be seen in Figure 5.2 most roots are partially embedded in soil and partially showing through the glass. Especially where the soil is well compacted and the soil is in good contact with the surface of the glass the roots also have a good contact with the soil. In places the roots even disappear from view as they are totally covered by soil (Figure 5.2, arrows A and B). Where the soil is badly compacted air pockets have formed and when roots grow through these the roots have no contact with the soil (Figure 5.2, arrows C).

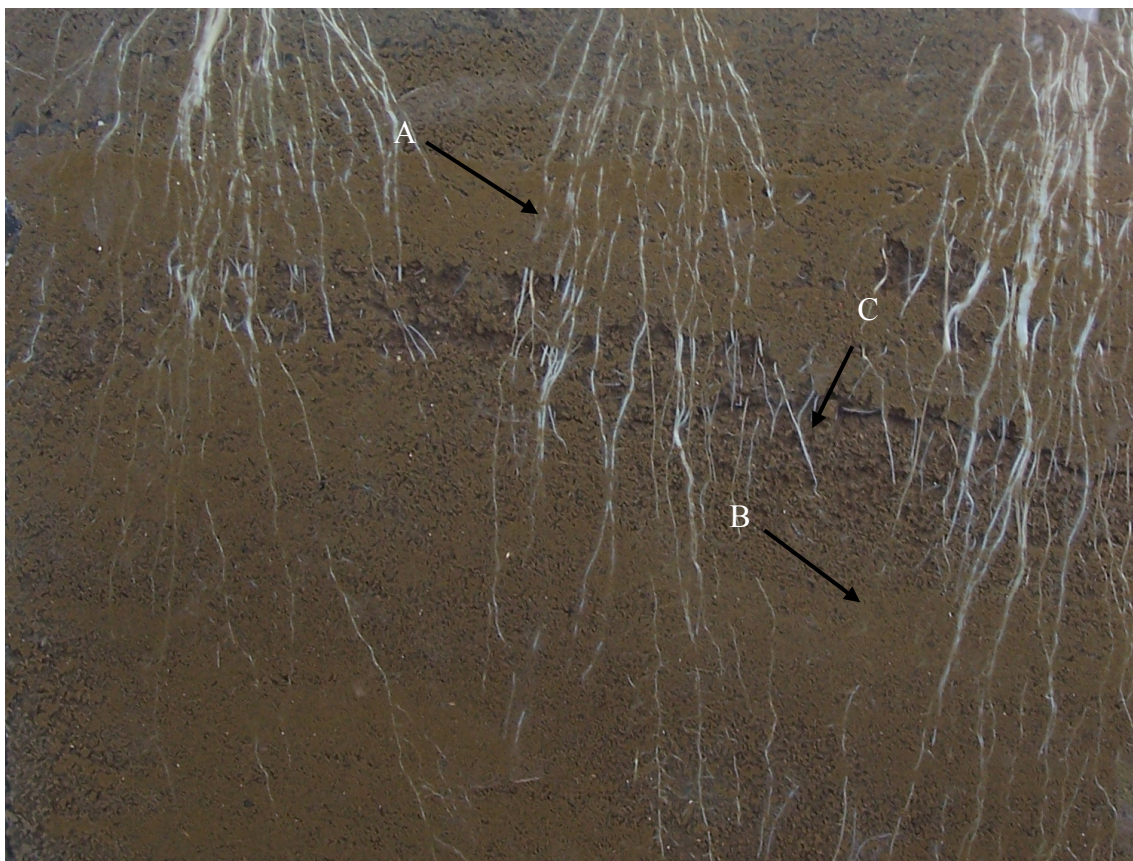


Figure 5.2 Close up of a control showing the roots in good contact with the soil (A and B) and sections with limited contact (C).

The method of using rhizoboxes will affect the three dimensional growth pattern since the method forces the roots to virtually only grow in two dimensions. It also affects the remediation potential because of the lower root/soil interface compared with a three dimensional system such as a pot trial or a rhizobox at 0° angle. These two factors are limitations of the method but are considered to be acceptable in that the benefits of actually being able to observe root growth are greater.

5.6.2. Heat effects

All continuous data concerning plant development show a levelling off in growth or even a reduction at one stage. This can be related to the weather. For approximately two weeks the temperature in the greenhouse, where the experiment was conducted, showed top readings of 45 °C and relatively high humidity though the humidity was not measured. This is a temperature where most plants are severely heat stressed. In the case of ample supply of water the shoots are able to keep a lower temperature thanks to the cooling effect of evaporation. However, high humidity reduces evaporation and plant growth is severely affected (Taiz & Zwiger, 2002). The plants were thus not only stressed by the treatments imposed on them, but also by heat. This might have caused some disturbances in the results. Heat also has an impact on microbial activity. Degradation of organics in soil has been seen to double for every increased 10 °C (Frick et al., 1999) which might have been a contributing factor to the high level of dissipation (approximately 80 %) during the time of the experiment. This level of

degradation can be compared with a study by Kaimi et al. (2004) which achieved a similar decrease in diesel concentration with aid of *L. perenne* after 3 months compared with 1 month in this study.

6. CONCLUSIONS

This study aimed at investigating the effects on root development of *L. perenne* in a highly diesel contaminated soil as well as root behaviour in response to spatial geometry of the contaminant. It has shown that by spreading a layer of fresh soil on top of a diesel contaminated layer germination is facilitated where the diesel contamination otherwise is too high to allow this, but that plant development of *L. perenne* is severely effected by the presence of diesel with a reduction in both root and shoot growth. Investigating the effect of layer geometry revealed no preferential roots growth in the path of clean soil in the treatment with a Discontinuous contamination layer. However the thickness of the clean soil covering the contamination was shown to have a great impact on root behaviour and plant performance. With a 5 cm thick layer of clean soil there is enough moisture and nutrients in this layer to sustain plant development without the roots having to penetrate the contaminated layer, but spread in the surface layer above it. A thinner layer of only 2.5 cm does not contain as much moisture and the roots are forced to grow through the contamination and thus show a stronger toxic response with a lower plant development.

The rate of diesel degradation showed a positive relationship with root development. The vegetated treatments that produced the highest root length also had a higher TPH dissipation rate though only the dissipation rate for the treatment with a thin layer of clean soil was significantly higher than non-vegetated or very poorly vegetated treatments. The importance of this observation is that improved hydrocarbon dissipation by roots is dependent on the position of the roots in relation to the contaminant. Roots have to be present inside the contamination for phytoremediation to be effective.

Sub-irrigation proved to provide insufficient water to the plants which caused very poor plant development. Neither could any movement of diesel, either up or down the profile, be detected despite whether irrigated from above or below.

The experiment with textural differentiation showed the importance of optimal moisture conditions for root and plant development with root spreading mainly in the finer textured layer regardless of diesel concentration.

Spreading a layer of clean soil on a diesel contaminated site does improve germination rate but creates other obstacles because roots show a tendency to preferential growth. If roots avoid penetrating the contamination the beneficial effect on hydrocarbon degradation is lost. Mixing the clean soil by tillage with the contaminated soil and thereby lowering the average concentration could be a viable option. A lower concentration would facilitate germination and decrease plant toxicity without allowing roots preferential growth. However, this would increase the volume of contaminated soil and possibly the depth of contamination. This study has also drawn attention to the importance of maintaining good physical conditions, like moisture content, for optimal plant growth which is crucial for effective phytoremediation.

6.1. Further research

With a longer experimental period the relationship between root development and behaviour, and TPH dissipation should become more evident as the roots develop and the contamination decreases. As the roots continue to develop in the heterogeneously contaminated soil is also of interest to study the further root behaviour in relation to the location of contamination. Therefore it is recommended that if further studies of this kind are undertaken it should have duration of at least three months to fully see these effects.

With additional treatments such as varying layer thickness and contamination concentrations, more extensive conclusions of root behaviour could be made. It is also recommended a better design of the sub-irrigation system is tested to fully be able to draw any worthwhile conclusions from this treatment.

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APPENDIX 1

Statistical analysis of significant differences between treatments

The content of appendix 1 is tables of means and differences of means to establish significant differences between treatments over time. These were obtained from using ANOVA in GenStat.

Shoot height

Table A1. Average shoot height (cm) over time for the different treatments

Time (days)	Control	Dis-continued	Normal	Sub-irrigation	Sand Control	Sand Low	Sand High
1	4.9	4.3	2.9	3.0	4.9	6.1	6.2
4	6.7	5.5	3.8	3.0	6.4	7.3	6.8
7	8.3	6.1	4.6	3.5	8.7	8.0	7.2
10	10.6	6.8	4.9	3.6	9.9	8.8	7.6
13	11.2	6.7	4.4	2.7	10.3	8.8	7.9
16	12.2	6.6	4.3	2.6	10.5	8.7	7.9
22	11.1	6.1	4.5	2.5	10.2	8.8	7.9
25	10.6	5.9	4.6	2.4	10.6	8.2	7.5

Table A2. Differences of mean for shoot height over time (cm). Significant difference ($p = 0.05$) if larger than 0.55 (l.s.d), which are marked in *italic*

Time (days)	Control / Normal	Normal Sub-irrigation	Control Sub-irrigation	Control Discontinuous	Discontinuous / Normal	Discontinuous / Sub-irrigation
1	1.96	-0.14	1.83	0.58	1.38	1.245
4	2.89	0.85	3.73	1.21	1.67	2.522
7	4.05	0.72	4.77	2.20	1.84	2.563
10	5.69	1.27	6.96	3.74	1.95	3.219
13	6.82	1.64	8.46	4.52	2.30	3.937
16	7.87	1.66	9.54	5.51	2.36	4.022
22	6.61	1.96	8.57	4.95	1.66	3.623
25	6.00	2.19	8.19	4.73	1.28	3.467

Table A3. Differences of mean for shoot height (cm) over time in Sand experiment. Significant difference ($p = 0.05$) if larger than 0.95 (l.s.d), which are marked in *italic*

Time (days)	Control/ Low	Control / High	High / Low
1	-1.211	-1.366	-0.155
4	-0.889	-0.433	0.456
7	0.777	1.566	0.789
10	1.155	2.289	1.134
13	1.412	2.323	0.911
16	1.745	2.589	0.844
22	1.416	2.283	0.867
25	2.382	3.148	0.766

Shoot number

Table A4. Average shoot number over time for the different treatments

Time (days)	Control	Discontinuous	Normal	Sub-irrigation	Sand Control	Sand Low	Sand High
1	2.7	2.1	2.1	1.9	2.6	2.4	2.4
4	3.6	2.7	2.3	1.9	3.2	3.2	2.8
7	4.3	2.9	2.8	2.0	4.2	3.4	3.1
10	5.4	3.2	2.9	1.9	5.3	4.0	3.3
13	6.2	2.8	2.1	1.3	5.6	3.7	3.2
16	7.0	2.4	2.0	1.2	6.2	3.2	2.9
22	11.4	3.0	2.4	1.4	8.3	4.8	3.7
25	15.0	3.6	3.0	1.6	9.3	5.2	4.0

Table A5. Differences of mean for shoot number over time. Significant difference ($p = 0.05$) if larger than 0.54 (l.s.d), which are marked in *italic*

Time (days)	Control / Normal	Normal Sub-irrigation	Control / Sub-irrigation	Control Discontinuous	Discontinuous / Normal	Discontinuous / Sub-irrigation
1	0.56	0.19	0.74	0.56	0.00	0.19
4	1.26	0.41	1.67	0.89	0.37	0.78
7	1.48	0.74	2.22	1.37	0.11	0.85
10	2.48	1.07	3.56	2.22	0.26	1.33
13	4.04	0.85	4.90	3.38	0.67	1.52
16	5.04	0.82	5.85	4.63	0.41	1.22
22	8.94	1.00	9.94	8.42	0.52	1.52
25	12.08	1.33	13.41	11.41	0.67	2.00

Table A6. Differences of mean for shoot number over time in Sand experiment. Significant difference ($p = 0.05$) if larger than 0.94 (l.s.d), which are marked in *italic*

Time (days)	Control/ Low	Control / High	High / Low
1	0.11	0.11	0.00
4	0.00	0.44	0.44
7	0.78	1.11	0.33
10	1.33	2.00	0.67
13	1.89	2.33	0.45
16	3.00	3.33	0.33
22	3.56	4.67	1.11
25	4.03	5.26	1.22

Shoot Health

Shoot health is calculated as highest shoot per plant (cm) multiplied with the number of shoots per plant.

Table A7. Average Shoot health over time for the different treatments (cm)

Time (days)	Control	Discontinuous	Normal	Sub-irrigation	Sand Control	Sand Low	Sand High
1	14.3	9.8	6.4	6.1	12.8	15.1	15.6
4	26.4	16.2	9.7	7.5	21	23.8	19
7	39.3	19.4	13	10.1	37.8	27.6	22.4
10	63.9	24.6	15.5	9.8	53.7	34.8	25.5
13	79.5	23.5	14.7	7.6	57.4	32.5	26.1
16	86.4	20.5	14.4	7.5	66.8	28.4	23.3
22	147.4	27	18.7	8.3	85.4	41.2	29.5
25	185.2	31.8	24.5	10.1	101.1	42.4	30.7

Table A8. Differences of mean for shoot health (cm) over time. Significant difference ($p = 0.05$) if larger than 8.56 (l.s.d), which are marked in *italic*

Time (days)	Normal / Control	Normal / Sub-irrigation	Control / Sub-irrigation	Discontinuous / Control	Discontinuous/ Normal	Discontinuous / Sub-irrigation
1	7.9	0.3	8.2	4.5	3.4	3.7
4	16.7	2.2	18.9	10.2	6.5	8.7
7	26.3	2.9	29.2	19.9	6.4	9.3
10	48.4	5.7	54.1	39.3	9.1	14.8
13	64.8	7.1	71.9	56	8.8	15.9
16	72	6.9	78.9	65.9	6.1	13
22	128.7	10.4	139.1	120.4	8.3	18.7
25	160.7	14.4	175.1	153.4	7.3	21.7

Table A9. Differences of mean for shoot health (cm) over time in Sand experiment. Significant difference ($p = 0.05$) if larger than 14.8 (l.s.d), which are marked in *italic*

Time (days)	Control/ Low	Control / High	Low / High
1	-2.3	-2.8	-0.5
4	-2.8	2	4.8
7	10.2	15.4	5.2
10	18.9	28.2	9.3
13	24.9	31.3	6.4
16	38.4	43.5	5.1
22	44.2	55.9	11.7
25	58.7	70.4	11.7

Root Development

Table A10. Average root lengths over time for derived from root drawings (cm)

Time (days)	Normal	Discontinuous	Sub-irrigation	Control	Sand Control	Sand Low	Sand High
1	6.13	24.86	8.21	47.93	1.94	1.09	4.89
4	14.51	56.96	10.57	105.32	35.43	13.56	39.29
7	27.03	83.23	14.44	227.81	110.49	42.11	78.75
11	55.36	121.00	28.07	396.55	150.16	60.29	134.11
15	67.91	132.72	32.54	456.96	186.27	72.21	149.16
20	69.48	136.16	34.06	454.94	195.06	78.03	149.96
23	81.26	146.34	34.36	498.00	263.31	90.78	176.71
27	126.09	203.51	35.84	769.20	485.27	150.72	270.33

Table A11. Differences of mean for root length (cm) over time. Significant difference ($p = 0.05$) if larger than 29.95 (l.s.d), which are marked in *italic*

Time (days)	Discontinuous / Normal	Normal / Sub-irrigation	Control / Normal	Discontinuous / Sub-irrigation	Control/ Discontinuous	Control / Sub-irrigation
1	18.73	-2.08	41.81	16.65	23.08	39.72
4	42.45	3.94	90.81	46.39	48.36	94.75
7	56.20	12.59	200.78	68.79	144.58	213.37
11	65.64	27.29	341.19	92.92	275.55	368.47
15	64.81	35.38	389.05	100.19	324.24	424.43
20	66.68	35.42	385.46	102.10	318.78	420.88
23	65.09	46.90	416.74	111.99	351.65	463.64
27	77.41	90.26	643.11	167.67	565.69	733.36

Table A12. Differences of mean for root length (cm) over time in Sand experiment. Significant difference ($p = 0.05$) if larger than 51.87 (l.s.d), which are marked in *italic*

Time (days)	Sand Control / Sand Low	Sand Control / Sand High	Sand High / Sand Low
1	0.85	-2.95	3.79
4	21.87	-3.86	25.73
7	68.37	31.73	36.64
11	89.87	16.05	73.82
15	114.06	37.11	76.94
20	117.03	45.10	71.93
23	172.52	86.59	85.93
27	334.55	214.95	119.60

Biomass

Table A13. Treatment means for shoot and root dry biomass (g)

	Normal	Discontinuous	Sub-irrigation	Control	Sand Control	Sand Low	Sand High
Shoot	0.013	0.030	0.009	0.164	0.108	0.051	0.034
Root	0.0224	0.0246	0.0022	0.1169	0.0752	0.0372	0.0352

Table A14. Differences of mean for dry Biomass (g) at time of harvest for the different treatments. Significant differences are marked in *italic* (larger than l.s.d)

	I.s.d	Discontinuous / Normal	Discontinuous / Sub-irrigation	Control / Discontinuous	Control / Sub-irrigation	Control / Normal	Normal / Sub-irrigation	Control / Low	Control / High	High / Low
Shoot	0.0421	0.0178	0.0211	0.1339	0.155	0.1517	0.0033	0.056	0.073	0.017
Root	0.035	0.002	0.022	0.092	0.115	0.095	0.020	0.038	0.04	0.002

TPH (Total Petroleum Hydrocarbon)

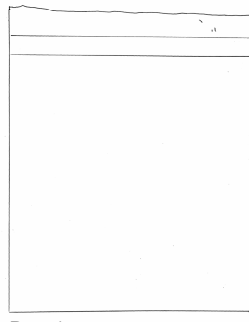
Table A15. Differences of mean for TPH (mg/g). Significant differences are marked in *italic* (larger than l.s.d). For comparison with Control within the Surface layer, lsd is 0.0595 but 0.0652 in other cases

	I.s.d	Discontinuous / Normal	Discontinuous / Sub-irrigation	No Discontinuous Plants	Discontinuous / Control	No Plants / Control	Sub-irrigation / Normal	Sub-irrigation / No Plants	Sub-irrigation / Control	Normal / No Plants	Normal / Control
Contaminated	0.481	0.164	-0.419	0.56	*	*	0.583	-0.125	*	-0.708	*
Surface	0.0652/ 0.0595	-0.010	-0.077	-0.055	-0.007	0.015	0.067	0.055	0.070	-0.012	0.003
Sub-layer	0.0446	-0.099	-0.023	0.024	0.014	0.061	-0.076	-0.024	0.037	0.052	0.113

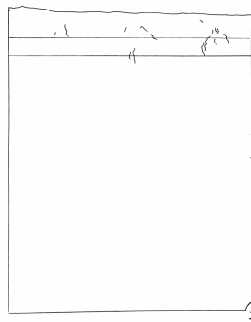
APPENDIX 2

Root drawings

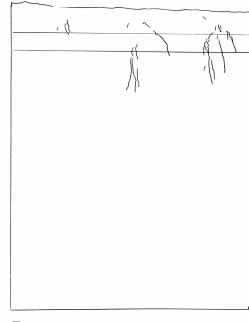
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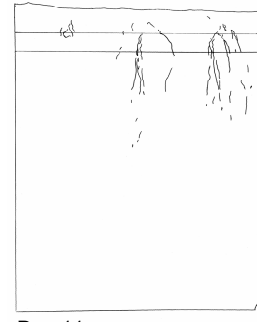
Day 1



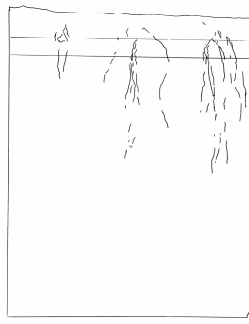
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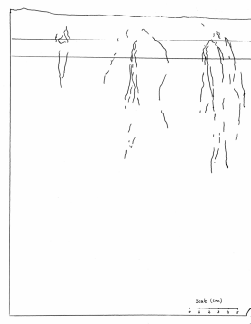
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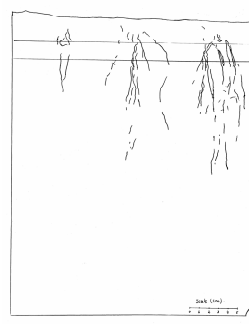
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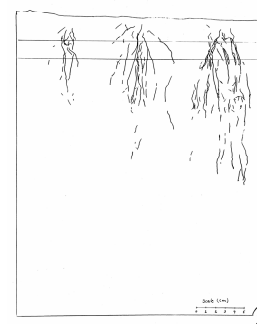
Day 15



Day 20

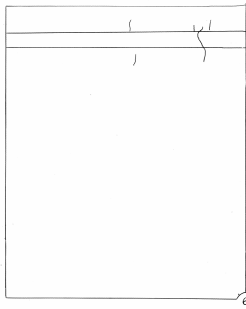


Day 23

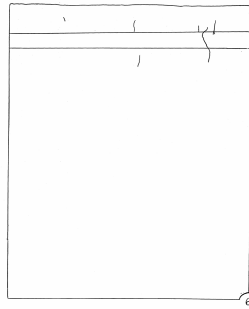


Day 27

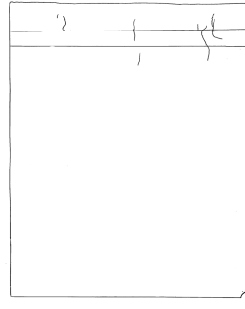
Normal
Box 6



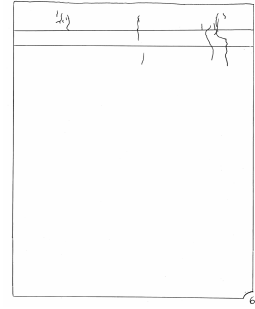
Day 1



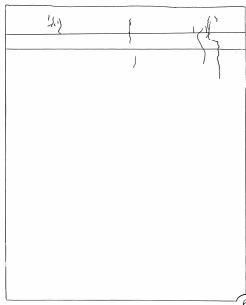
Day 4



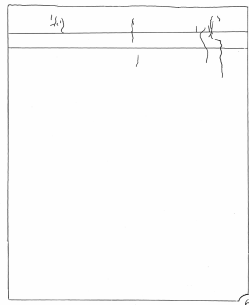
Day 7



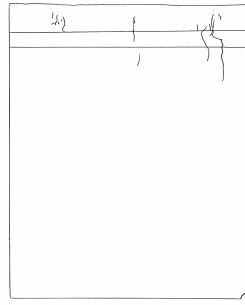
Day 11



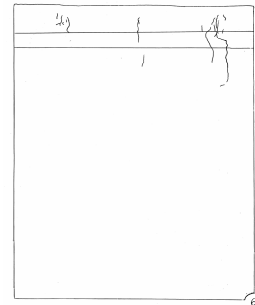
Day 15



Day 20

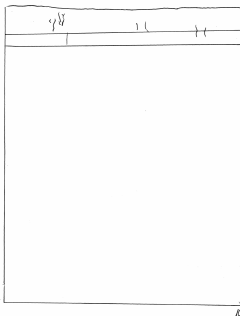


Day 23

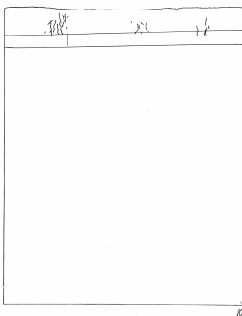


Day 27

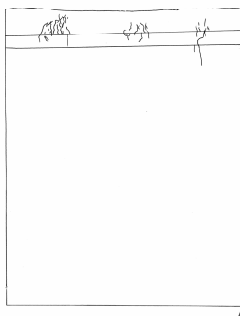
Normal
Box 10



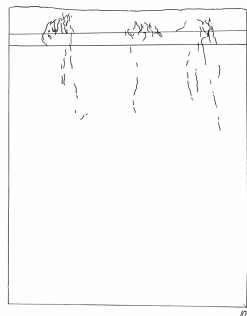
Day 1



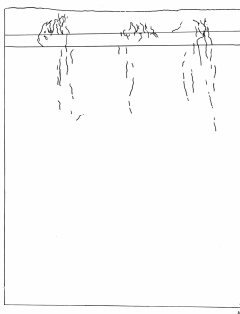
Day 4



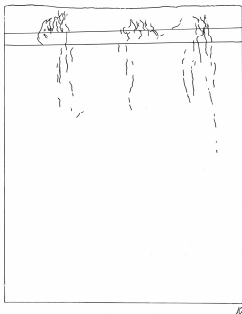
Day 7



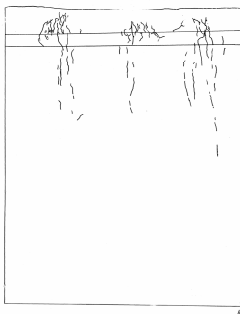
Day 11



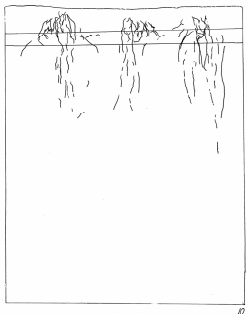
Day 15



Day 20

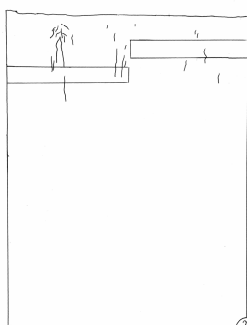


Day 23

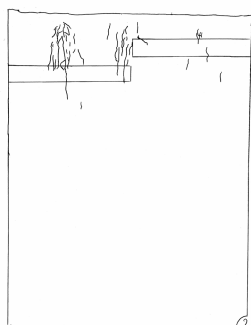


Day 27

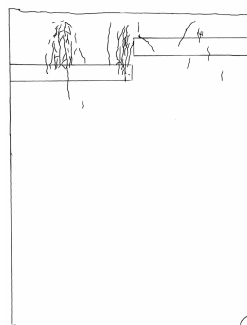
Discontinuous Box 2



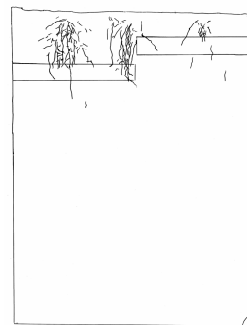
Day 1



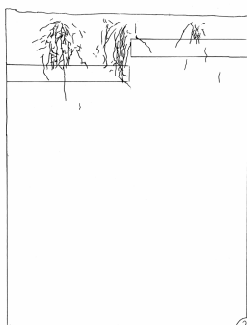
Day 4



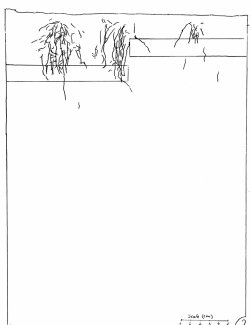
Day 7



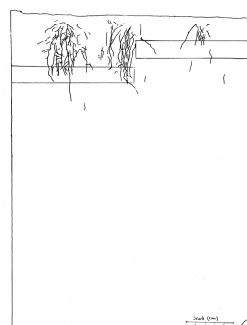
Day 11



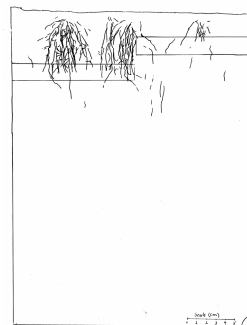
Day 15



Day 20

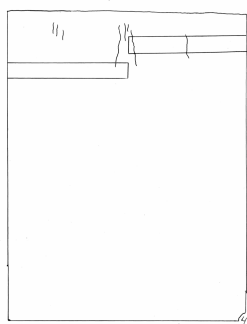


Day 23

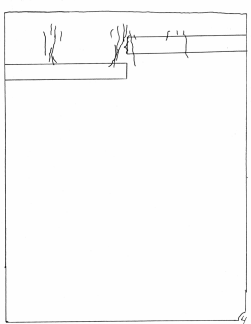


Day 27

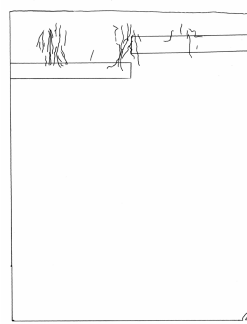
Discontinuous Box 4



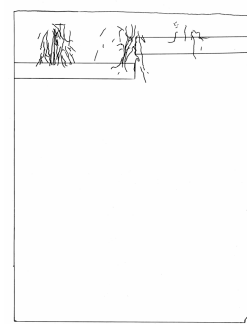
Day 1



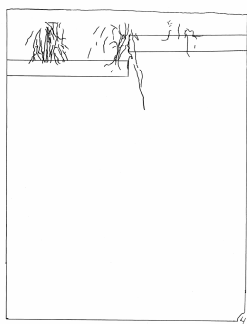
Day 4



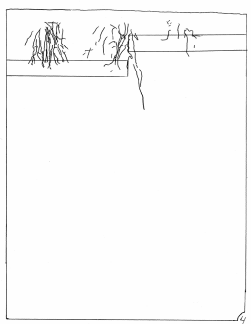
Day 7



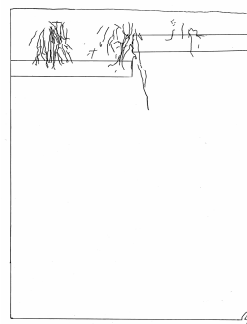
Day 11



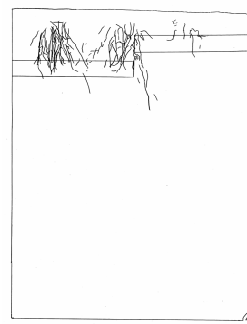
Day 15



Day 20

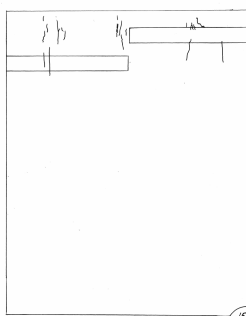


Day 23

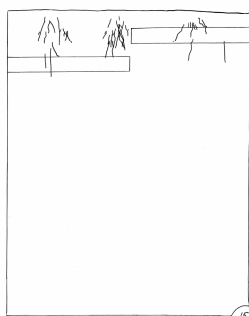


Day 27

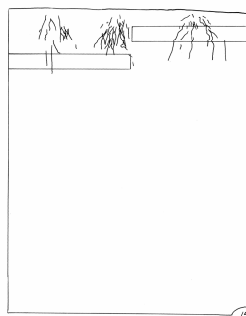
Discontinuous Box 15



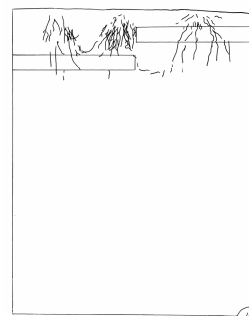
Day 1



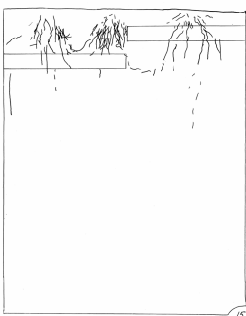
Day 4



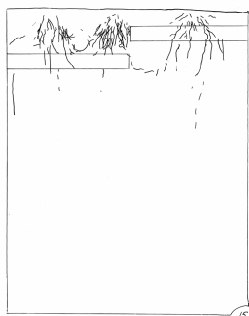
Day 7



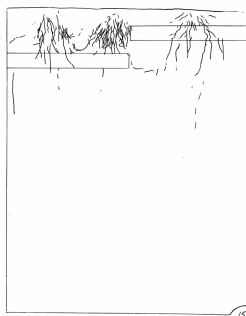
Day 11



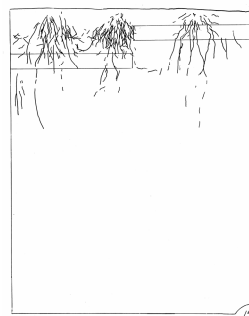
Day 15



Day 20

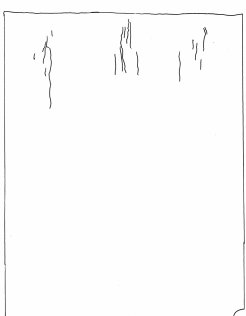


Day 23

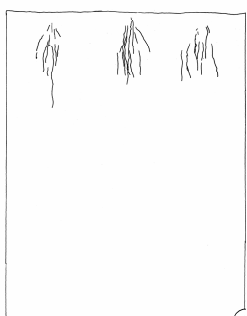


Day 27

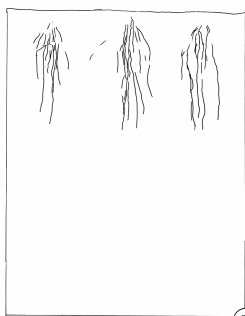
Control Box 5



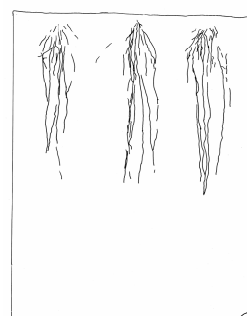
Day 1



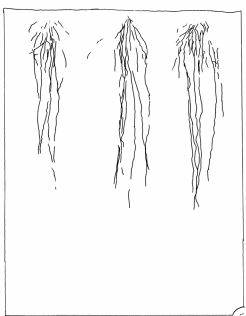
Day 4



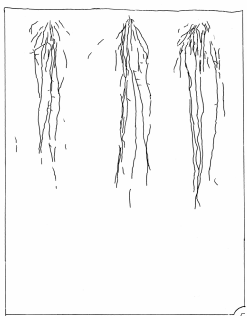
Day 7



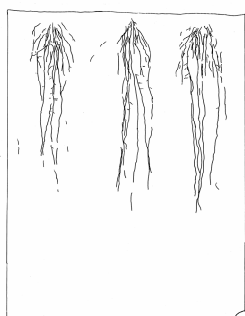
Day 11



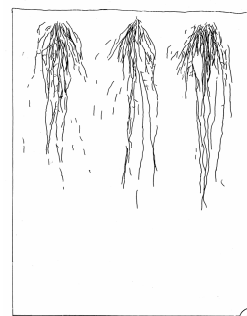
Day 15



Day 20

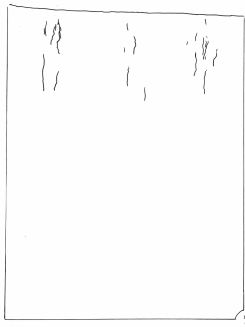


Day 23

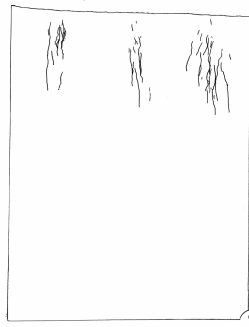


Day 27

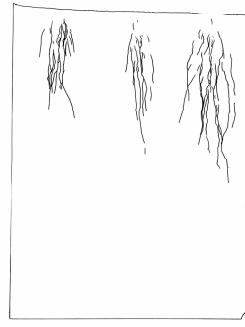
Control
Box 9



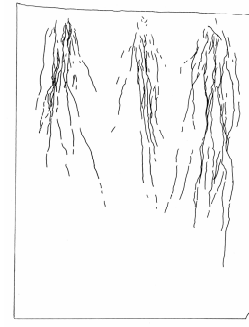
Day 1



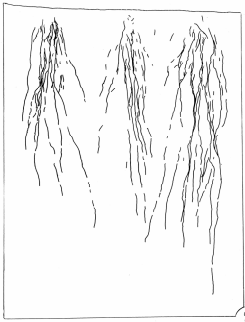
Day 4



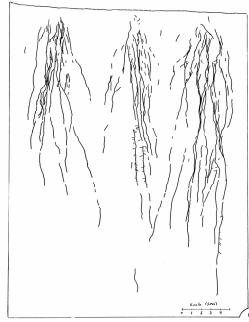
Day 7



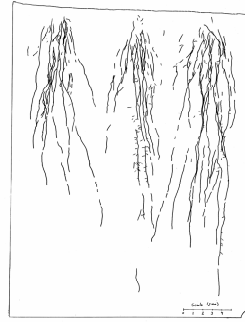
Day 11



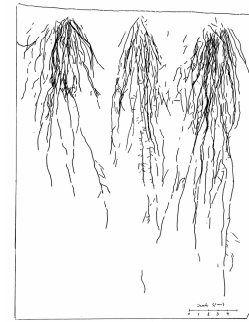
Day 15



Day 20

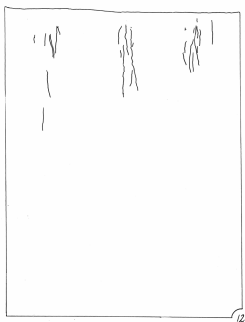


Day 23

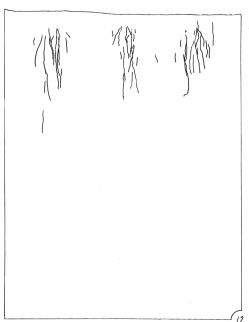


Day 27

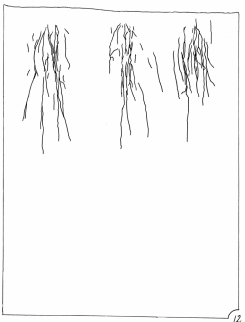
Control
Box 12



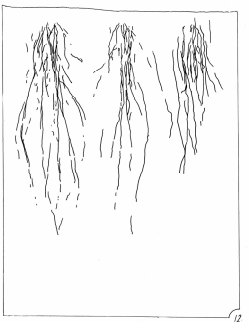
Day 1



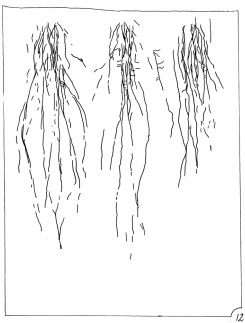
Day 4



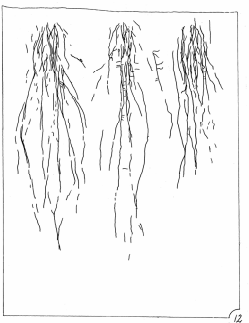
Day 7



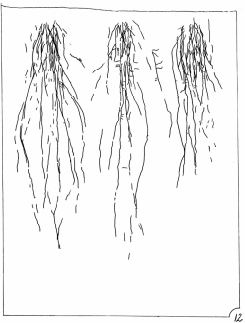
Day 11



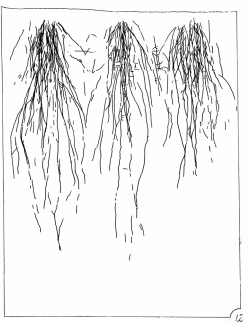
Day 15



Day 20

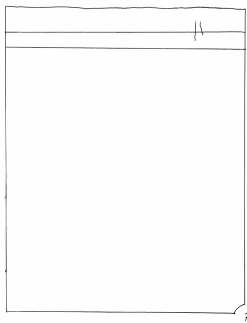


Day 23

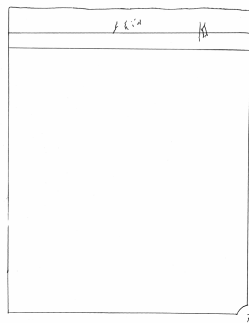


Day 27

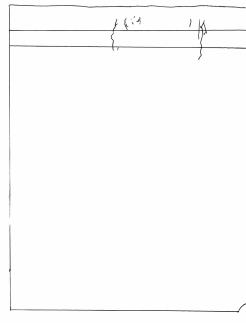
Sub-irrigation Box 7



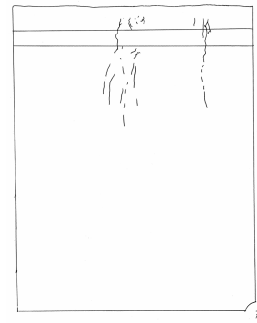
Day 1



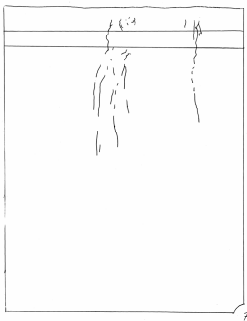
Day 4



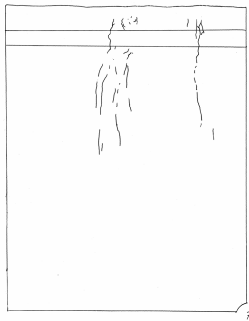
Day 7



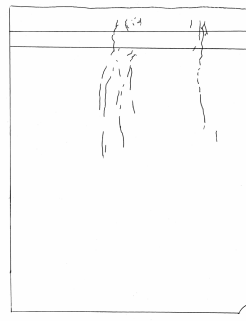
Day 11



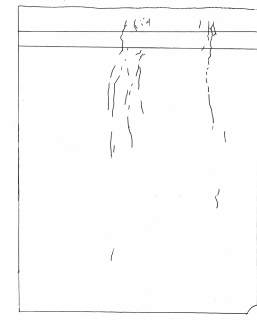
Day 15



Day 20

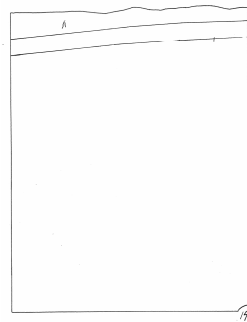


Day 23

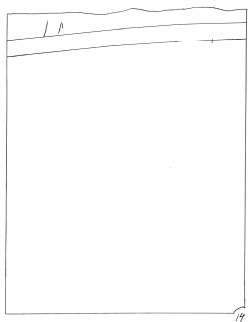


Day 27

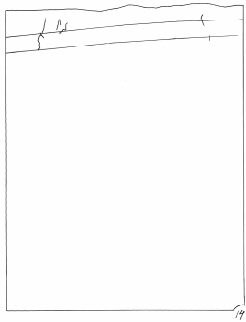
Sub-irrigation Box 14



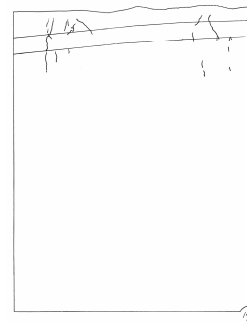
Day 1



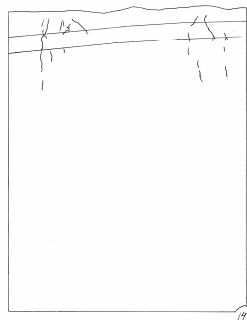
Day 4



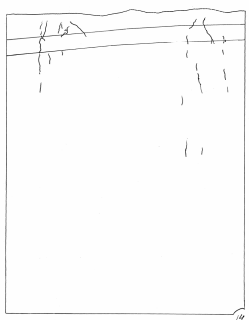
Day 7



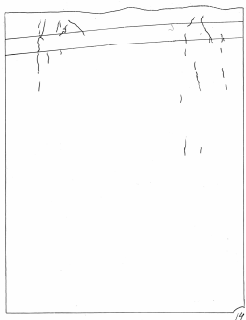
Day 11



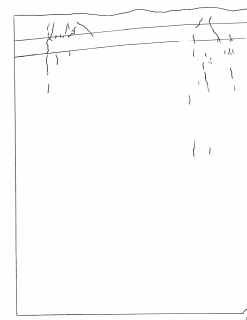
Day 15



Day 20

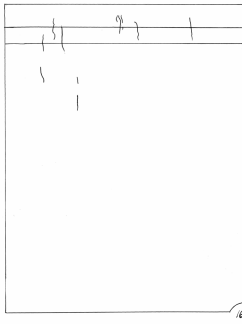


Day 23

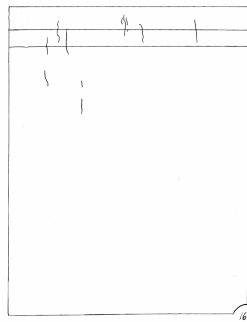


Day 27

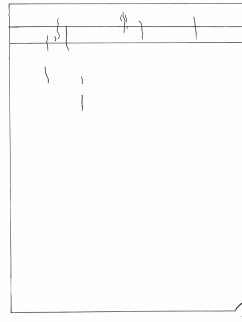
Sub-irrigation Box 16



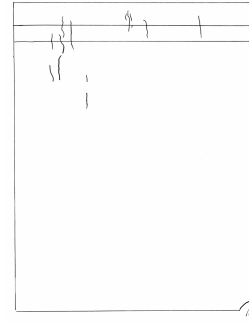
Day 1



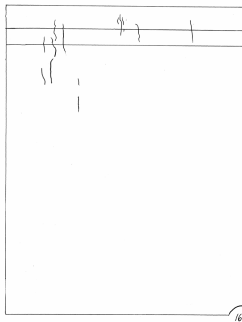
Day 4



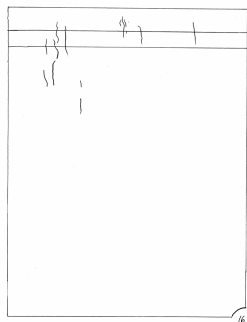
Day 7



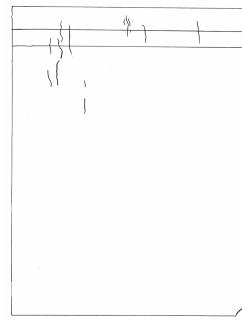
Day 11



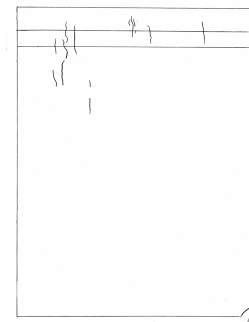
Day 15



Day 20

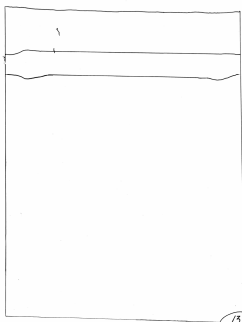


Day 23

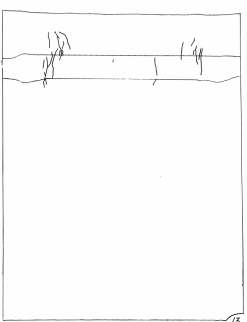


Day 27

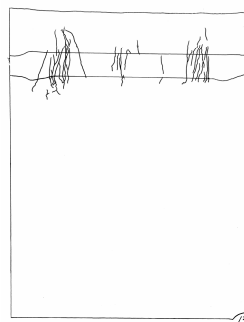
Sand Control Box 13



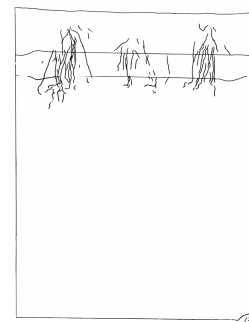
Day 1



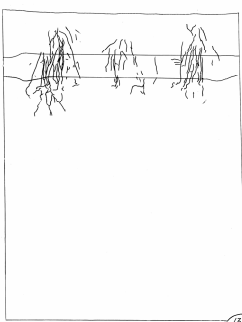
Day 4



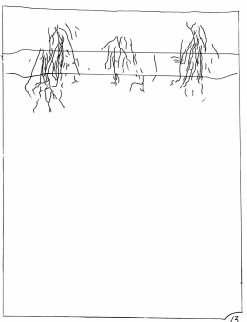
Day 7



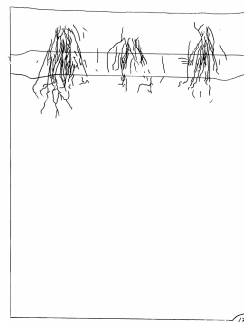
Day 11



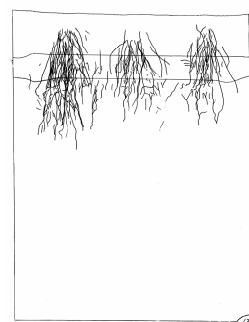
Day 15



Day 20

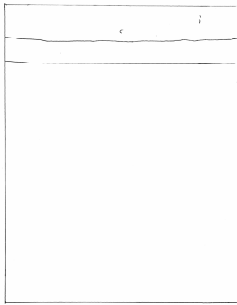


Day 23

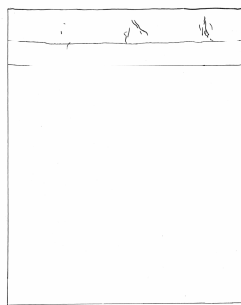


Day 27

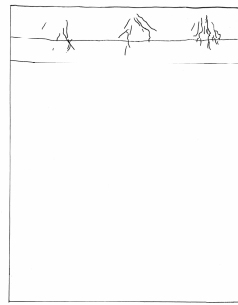
Sand Low Box 17



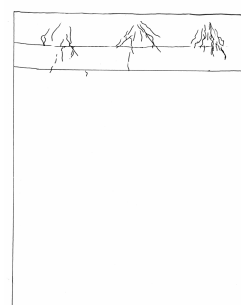
Day 1



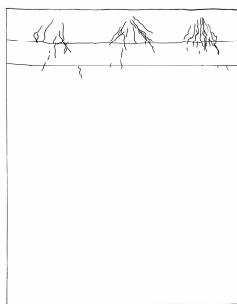
Day 4



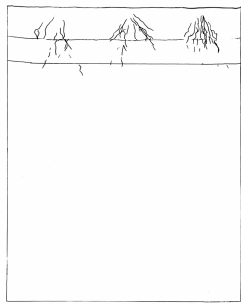
Day 7



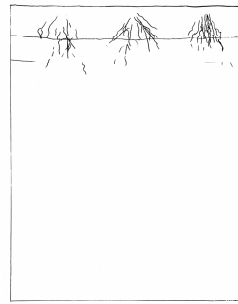
Day 11



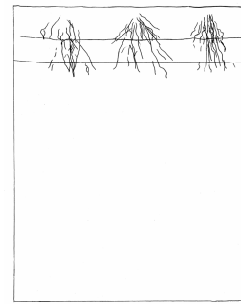
Day 15



Day 20

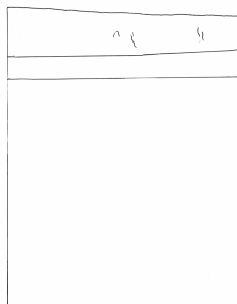


Day 23

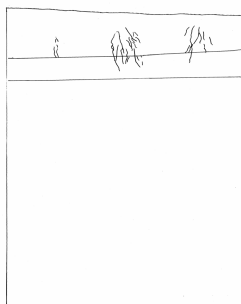


Day 27

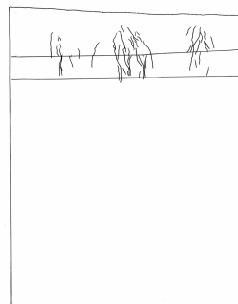
Sand High Box 11



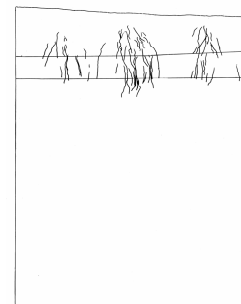
Day 1



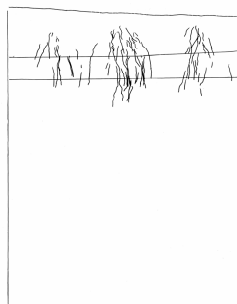
Day 4



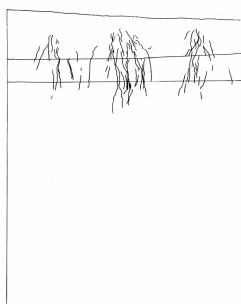
Day 7



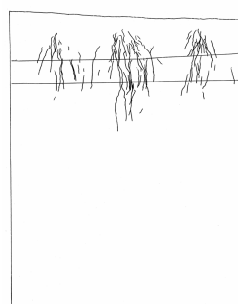
Day 11



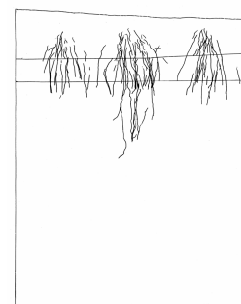
Day 15



Day 20



Day 23



Day 27

Förteckning över utgivna nummer i rapportserien (ISSN 1653-6797). 2006 -

List of publications in the Report series (ISSN 1653-6797). 2006 -

- 1 Anna Krafft. 2006. The effect of urban runoff on the water quality of the Sweetbriar Brook, Ampthill, UK. (Dagvattnets effekt på vattenkvaliteten i Sweetbriar Brook, Ampthill, Storbritannien). 66 p.
- 2 Karin Pettersson. 2006. Root development of *Lolium Perenne* in diesel contaminated soil. (Rotutveckling hos *Lolium Perenne* i dieselkontaminerad jord). 54 p.

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